EDITORIAL REVIEW

CARL von VOIT

Carl Voit was born in Amberg, Bavaria, October 31, 1831. He was the son of a well-known architect, August Voit, who later in 1854 designed the Crystal Palace in Munich, the locale of his son's life work. After completing his work in the gymnasium, Carl applied himself in 1848 to the study of medicine at the University of Munich. In 1851 to 1852 he attended the University of Würzburg, which enjoyed the highest scholastic reputation in Germany at that time. Here he listened to lectures by such renowned men as Köllicker, Leydig, Virchow, Skanzoni and Scherer. In 1852 he was back at Munich, where in the meantime Liebig and Pfeufer had been called, initiating a new scientific life in the Hochschule. Completing his medical course in 1854, Voit continued his studies in the natural sciences under the physicist Jolly, the zoölogist Siebold, the anatomist and physiologist Bischoff and the chemist Liebig.

On the advice of Liebig, in 1855 Voit went to Göttingen, where he spent a most inspiring year in Wöhler's laboratory. Here he made a special study of benzoyl compounds and, under Listing, acquired an unusual knowledge of physiological optics.

In 1856 Voit was appointed Privat-dozent in the Physiological Institute of Munich, becoming an assistant to Pettenkofer, professor of medical chemistry. Here he published his first scientific work, a paper entitled: "Untersuchungen von Bodenarten zur Entscheidung pflanzen physiologischer Fragen." This was followed shortly by a paper "Über die Aufnahme von Quecksilber und seiner Verbindungen in den

Körper," and by the noteworthy paper, published with Bischoff, "Die Gesetze der Ernährung des Fleischfressers," in 1860.

In 1859 he declined a call to Tübingen. On the death of Harless, professor of physiology at Munich, in 1863, Voit succeeded to his chair and also to the directorship of the Physiological Institute, positions that he held until his death on January 31, 1908. His renown brought him many honors, including election to membership in the Academy of Sciences in 1865. In the same year, he with Buhl, Pettenkofer and Radlkofer founded the Zeitschrift für Biologie.

At 55, when Lusk first knew Voit, he was "alert, with a quick walk, of quiet, dignified, courteous bearing." He was as simple as a German of the old school. He never attended scientific meetings except those of the local scientific society, which were social in nature. "Only through his Zeitschrift für Biologie did he rub shoulders with the world." He attracted many students and possessed the quality of holding their affection and loyalty. If judged only by the performance of those who received their training in his laboratory, his influence on the development of the science of nutrition was enormous. Among his students are numbered Rubner, Prausnitz, Atwater, Lusk, Y. Henderson, E. Voit, Cathcart, Max Cremer, Otto Frank, who succeeded him as director of the Physiological Institute, and many others of note.

Carl Voit is often referred to as the founder of metabolic research. While Liebig opened a new era in the science of nutrition—the quantitative and therefore the scientific era—he was essentially a chemist, not a biologist. He was a great experimentalist but his theories of nutrition and metabolism were not based upon experiment: in fact, he never performed an animal experiment. On the contrary, Voit was well grounded in biology, as well as the other natural sciences, and developed a refined experimental technic for the study of animal physiology. While Liebig elaborated chemical methods for the searching analysis of animal tissues and excretions, Voit applied these methods to well-controlled

metabolism studies of the income and outgo of animals and of men. With painstaking care and an attention to experimental details that excited the admiration of his colleagues, he elaborated a methodology in metabolic research that was a model for others to follow. As Lusk has said, "*Genauigkeit* was the watchword of his laboratory." Supremely critical of the work of others, he was consistent in being as critical of his own work. Any uncertainty concerning the accuracy of a method of analysis, an analytical result, or an excretory collection was sufficient basis for discarding an experiment.

At the very beginning of his career, he believed that only by rigid methods of experimental control could the principles of animal metabolism be uncovered. Because of the inspiration he received from Wöhler, Liebig, Bischoff and Pettenkofer, and the interest he felt in the well-known work of Bidder and Schmidt at Dorpat, he turned first to the determination of the metabolism of protein, by following the income and outgo of nitrogen.

With the exception of the work of Bidder and Schmidt, previous experiments in this field had yielded little useful information, because, no matter what the protein intake might be, nitrogen equilibrium could not be demonstrated. There always remained a bothersome 'nitrogen deficit' that was quite commonly ascribed to a metabolism of gaseous nitrogen. It was presumed that some volatile substance containing nitrogen, such as ammonia, was expired through the lungs, and even that atmospheric nitrogen entered into the animal economy. Even his master, Bischoff, had been unable to attain nitrogen equilibrium in animal experiments.

With Bischoff, and with his new methods of experimentation, Voit immediately obtained results of the greatest importance. The troublesome 'nitrogen deficit' disappeared. In experiments on dogs, and later in a 124-day metabolism experiment on a pigeon, it was shown that on adequate feeding the nitrogen of the solid and liquid excreta equals the nitrogen consumed, and that this condition of nitrogen equilibrium is always obtained with adult animals on constant

feeding inside very wide limits of nitrogen intake. The 'nitrogen deficit' was evidently the child of inaccurate analytical methods.

This finding, together with the later determination that the losses of nitrogen through hair growth, epidermis and sweat secretion is generally negligible, enormously simplified the technic of protein studies, which consequently are concerned only with the nitrogen content of food, feces and urine, except for special problems. The importance of the establishment of a condition of nitrogen equilibrium to studies of the effects of any factor on protein metabolism is obvious.

The protein metabolism studies of Bischoff and Voit on dogs uncovered many other important facts. They showed that in fasting there is always a considerable nitrogen loss; that the consumption of meat leads to an equilibrium of intake and outgo of nitrogen, which may be reestablished at any higher meat intake after the lapse of a sufficient adjustment period; that the giving of fat or carbohydrate to an animal in nitrogen equilibrium decreased the loss of nitrogen. In particular, it was shown that muscular work does not increase the nitrogen output of a dog receiving an adequate intake of non-nitrogenous nutrients, a finding that was confirmed later on human subjects by Ranke, a pupil of Voit, and later still by Voit himself. These studies, together with the contemporary observations of Fick and Wislicenus in 1865 made during their ascent of the Faulhorn, and particularly the more complete observations of N. Zuntz during his ascent of the much higher Monte Rosa in 1906, sounded the death knell for the theory of Liebig that the energy for muscular work could be derived only from the decomposition of muscle tissue itself. Liebig based his whole theory of nutrition upon the unique value of dietary protein in replacing these muscle losses, as well as the losses of glandular substance during the process of secretion.

With Bischoff, Voit developed the conception of feces formation that is valid today. They observed that feces were formed during fasting and that during nutrition on highly

digestible diets the feces formed were not proportional in amount to the food consumed. Hence, they consist largely of the unabsorbed residues of the digestive juices.

From studies on the metabolism of protein, Voit's interest naturally turned to the metabolism of carbohydrates and fats. But such an extension of his program required the construction of a respiration apparatus for the determination of the outgo of carbon and the income of oxygen. It was Pettenkofer's great contribution to design a chamber large enough to accommodate a man, equipped to permit continuous observations of the gaseous exchange over many hours, and ventilated by the open circuit method so that such protracted confinement would not become irksome. The apparatus was a great improvement over those theretofore used, and the life-long friendship of Pettenkofer and Voit gave to science a series of important facts and principles established by the use of this instrument of research. Later, with a modified respiration chamber, Voit continued on laboratory animals his investigations of the total metabolism. The computations made from the recorded measurements of the income and outgo of nitrogen and carbon and the consumption of oxygen were expressed not in calories, but in the amounts of protein, carbohydrate and fat stored and oxidized in metabolism.

Pettenkofer and Voit found that in starvation only protein and fat are burned; that during work more fat is oxidized, but that during rest fat is readily deposited in the body; that carbohydrates are completely burned no matter how much is given, in this manner protecting the stores of fat. They believed that the metabolizability of the nutrients in the body is not proportional to their combustibility outside the body. Thus, proteins are the most easily metabolizable but are combustible with difficulty in vitro. On the contrary, fats are difficultly metabolizable, but most readily combustible in the laboratory. Carbohydrates are intermediate in both respects.

While Voit's researches with Pettenkofer were not calorimetric studies in the modern conception of the term they afforded the impetus for the development of the modern

theories of the metabolism of energy. Rubner served his apprenticeship under Voit and later at Marburg he continued his brilliant work in his own laboratory, establishing the validity in the animal body of the law of the conservation of energy, defining the replaceability of the nutrients in metabolism, illuminating the regulatory mechanism for the maintenance of the body temperature of homeotherms, confirming the surface area law of basal energy expenditures, and measuring the specific dynamic effects of the nutrients. All these epoch-making researches had their inception in the laboratory of Voit at Munich.

Early in their association, Pettenkofer and Voit concerned themselves with the metabolism of disease—diabetes, leucemia, anemia, fever. However, since at that time clinics and hospitals were not provided with respiration apparatus as they are today, they were perforce restricted to studies of the metabolism of nitrogen. Comparisons were made with healthy people on the same intake of food.

True to the traditions of Liebig, Voit did not confine himself to the scientific investigation of animal metabolism. He sought also to solve the practical problems of nutrition by determining the nutritive requirements of men under different conditions of living. His main experimental subject was his faithful laboratory helper Pistel, who spent his days in cleaning laboratory glassware and in tending experimental animals. Pistel was a robust man, blessed with a robust appetite, and for this reason, it is said, Voit's well-known dietary standard for a healthy man at moderate work is generously high. However, Voit's dietary recommendations are also based upon an extensive series of observations of the food habits of soldiers, prisoners, physicians, vegetarians, fat people, under-nourished people, and many others. These endeavors of Voit in Volksernährung also have been greatly extended by his pupil Rubner.

For a man of average size, healthy and performing only moderate labor, Voit recommends a daily consumption of 118 gm. of protein, 500 gm. of carbohydrate and 56 gm. of fat.

Hard workers may require up to 150 gm. of protein, 35% of which should be in the form of meat, and up to 200 gm. of fat daily. He would never recommend a consumption of more than 500 gm. of carbohydrates. With moderate activity, at least 25% of the fat consumed should be 'choice,' i.e., derived from bacon, lard or butter. With severe activity, at least 33% of the fat intake should be thus derived. Voit was decidedly opposed to vegetarianism. He freely admitted that some men could do well on less than 118 gm. of protein daily, but such men are generally light in weight, weak of constitution or physically inactive. This quota of protein for a normal man might be lowered to 108 gm. if it is largely derived from animal sources. A maintenance diet for prisoners and inmates of public institutions may contain only 85 gm. of protein, 30 gm. of fat and 300 gm. of carbohydrates.

In his practical recommendations, Voit emphasized cannily (and soundly) the importance of palatability in food. In his mind a food is first of all a palatable mixture of nutrients. His discussions of condiments (Genussmittel), including tea, coffee, alcoholic beverages, tobacco, as well as flavoring and aromatic substances, are interesting.

Condiments have a different but no less important part to fulfill in nutrition than the nutrients themselves and are as necessary as the latter for the preparation of a food. Animals and men would not usually consume a mixture of pure nutrients, but would die. As with every activity of the body, the act of taking food must be associated with a suitable sensation. The action of condiments may be compared with the lubricant for a machine or the whip for a working horse. In such a manner also the condiments function for the processes of nutrition and perform an indispensable service, although they are not able to prevent a loss of substance from the body or by their decomposition to provide us with living strength: they give us not actual strength but at most the feeling of strength by their influence on the nervous system.

It is refreshing to discover a nutrition expert who recognizes the requirements of the palate as well as those of the body.

A man's contribution to science rests not only upon the facts he establishes by experiment, but also upon his ingenuity in explaining these facts and in incorporating them into the body of knowledge of which they should be integral parts. From such explanatory theories the laws and principles of science are ultimately evolved. Voit's logical mind carried him far in explaining his experimental observations. His theories of metabolism have served a purpose in stimulating research, and as they fail to square with the newer knowledge of nutrition, they have been discarded. But it sometimes happens that the propounder of a theory considers himself its champion and is the last to admit its failure. He and his colleagues and pupils constitute a 'school of thought' dedicated to defend a theory or a group of theories against all comers. Passions are aroused, animosities are engendered and as a consequence the progress of science is impeded. The more abstruse the theory and the less amenable it is to direct experimental inquiry, the more obstinate and dogmatic its defenders become. Voit was human, intensely so, and he is no exception to this rule. His controversies with Pflüger, and especially with his old master, Liebig, are not the most inspiring phases of his career.

Voit's work and writings have contributed immensely to the evolution of our ideas concerning the fundamental nutrients and their functions in the body. According to the oldest conception, dating back to Hippocrates but persisting as late as 1833 in the writings of Beaumont, there was one 'common alimentary principle' or 'universal nutrient substance.' The recognition of 'three great staminal principles' pervades the writings of Prout (1785–1850), but no special differences in the nutritive functions of these principles were seen. The French physiologist Magendie (1785–1855) was apparently the first to demonstrate the unlike nutritive properties of the three foremost groups of organic foodstuffs. He clearly distinguished between the nitrogenous and non-nitrogenous nutrient materials. Mulder (1802–1880) coined the term 'protein' and emphasized the unique importance of the substances so grouped. Liebig (1803–1880) went still further

and exalted the importance of protein: all the energy for muscular work, glandular work and cellular work of all kinds comes only from protein. The main function of nutrition is to replace the cellular protein thus destroyed. As Verworn expressed it as late as 1899, "the life processes consist in the metabolism of the proteins;" carbohydrates and fats merely furnish animal heat.

Voit's work and writings emphasize the interchangeability of the nutrients within limits, and the sparing of one nutrient by another because of the fact that they serve like functions. According to him, the ingestion of food serves primarily to prevent the loss of protein and fat from the tissues. He believed in the conversion of protein into sugar and into fat, although, strangely, he denied the possibility of the conversion of carbohydrate into fat, believing that the experimental evidence adduced in favor of this conversion, which is considered overwhelming today, could be explained better by assuming a sparing of body fat and dietary fat by the carbohydrate of the diet.

Voit's outstanding experiments on the effect of muscular work on protein metabolism and on the respiratory exchange of man and the lower animals supplied the most telling argument against the erroneous theory of Liebig, but so imbued was he with Liebig's ideas on this point that he attempted to reconcile this evidence with the theory by proposing a mechanism by which the muscle at rest stored up electrical energy that was drawn upon during activity. He has stated emphatically: "Es muss für alle Zeiten rechtig bleiben, dass nur die organische, stickstoffhaltige Substanz krafterzeugend ist und die Bewegungsphänomene hervorbringt, und dass die Oxydation der Fette und Kohlehydrate ausschiesslich zür Wärmeproduktion, niemals aber als Energiequelle dienen könne." Only many years later was he constrained to admit the inevitable conclusion.

The fact that the heavy worker consumes more protein than the sedentary worker, and that he himself recommends a protein intake graded according to the muscular work to be

done, Voit explains not on the basis that only protein furnishes energy for muscular work, but on the assumption that the hard worker possesses a greater muscular mass than the sedentary worker, and for the maintenance of the greater mass of muscle he needs more dietary protein.

Voit's distinction between organized and circulating protein explained completely the experimental facts that he himself unearthed. With some modification it may be reconciled with Folin's ideas of an endogenous and an exogenous protein metabolism, and perhaps the modification required is no more profound than that to which Folin's theory has itself been subjected since its enunciation in 1905.

When Voit started his researches the idea was prevalent that the oxygen supply determined the intensity of metabolism, oxygen being the 'prime mover,' the 'instigator,' of oxidation processes, combining with all substances in the blood with which it possessed an affinity. A most noteworthy contribution of Voit was to point out most definitely that metabolism is not proportional to the oxygen supply, but that the level of metabolism determines the oxygen consumption. The horse was thus put before the cart.

Voit's experimental methods are still of basic importance. His experimental results are still valid. His experimental theories have either become the established principles of nutrition or they have fallen by the wayside. Some appear to us fantastic, but they should all be judged not by the truth contained in them, but by the service they have performed in harmonizing the facts established at the time of their elaboration and in guiding research to the discovery of new truths. Judged in this light the theories of Voit have served their purposes well. Voit himself did not take his theories too seriously, for he has said: "Die Resultate eines richtig angestellten und richtig verwerteten Experimentes bleiben für alle Zeiten unumstösslich, während eine Theorie im Fortschreiten der Wissenschaft umgestossen werden kann."

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THE EFFECT OF ENTERIC-COATED PANCREATIN ON FAT AND PROTEIN DIGESTION OF DEPANCREATIZED DOGS

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It is well known that exclusion of pancreatic juice from the intestine disturbs greatly the digestion of fat and protein. Thus, removal of the pancreas or occlusion of its ducts in animals leads to the loss of large amounts of fat and nitrogen in the feces, to a rapid and marked loss of body weight, and ultimately to death unless special care and diets are given. The addition of fresh or dried pancreas to the diet checks the excessive waste of food and body tissue and permits the animal to live indefinitely.

Although pancreatin has been used clinically for many years in cases of enzymatic deficiencies of the pancreas, results with the usual preparations have not been very successful (Bastedo, '25). In vitro experiments have shown that many enzymes are rapidly inactivated or destroyed by other enzymes and by an excess of hydrogen or hydroxyl ions. That trypsin may be digested by pepsin has been reported by Long and Johnson ('13), by Long and Hull ('16), by Northrop and Kunitz ('32), by Kleiner and Tauber ('34), and others. Long and Johnson ('13) showed that while trypsin is not destroyed or weakened by the addition of 0.2% HCl, it is greatly weakened by the presence of the acid and pepsin. That pancreatic lipase and amylopsin are destroyed in vitro by acid was reported by Lisbonne ('33). Schulman and

Rideal ('33) found that pancreatic solutions are digested by pepsin at pH 2.0. Kleiner and Tauber ('34) subjected pepsin and trypsin to reciprocal digestion and demonstrated that at pH 2.0 pepsin completely digests trypsin, leaving the pepsin unchanged. At this pH trypsin is inactive but not destroyed. If, however, a mixture of the two enzymes is kept at pH 5.5, which does not permit peptic activity, the pepsin is digested by trypsin.

Based on the assumption that the enzymes of pancreatin are inactivated in vivo by gastric juice, Sansum ('26, '32) has advocated the use of enteric-coated pancreatin granules and tablets in the treatment of certain forms of indigestion. The coating of these preparations presumably protects the contents from the destructive effect of gastric digestion, yet allows for immediate dissolution of the active enzymes in the intestine.

The present absorption studies were undertaken to determine the efficacy of enteric-coated pancreatin preparations in animals in which pancreatic juice is entirely excluded from the intestine by removal of the pancreas. Depancreatized dogs develop a greatly improved gastric digestion, and an increased gastric and intestinal acidity (Yesko, '28, and Fauley and Ivy, '29). These animals, therefore, make excellent experimental subjects for testing the hypothesis that pancreatic enzymes are inactivated by gastric juice and for testing the efficacy of enteric-coated preparations of pancreatin.

METHODS

Totally depancreatized dogs, weighing between 9 and 12 kg. and sustained by the administration of insulin, were fed 50 to 70 gm. of fresh beef per kilogram of body weight per day. In order to avoid marked fluctuations in absorption known to occur in depancreatized dogs and to obviate digestive disturbances, such as vomiting and diarrhea, only lean meat was fed. This highly palatable food was usually administered in three equal feedings, evenly spaced during the day. In later experiments only two feedings, at 8 A.M. and 8 P.M., were

given. The meat was thoroughly minced and mixed by grinding five or six times and was prepared in 80 pound lots and kept in tightly sealed jars at a temperature of 33°F. until used. The fat content of seven lots of meat prepared varied between 2.5 and 8.5%, the protein content between 18 and 21%.

Two preparations of pancreatin were used, small granules (0.5 to 1 mm. in diameter) and 5 grain tablets. Each was made up in two forms; one was coated, the other was not and thus served as control material. The pancreatin granules (both plain and enteric-coated) were preferred to the tablets since the former mix more thoroughly with the gastric contents and on passing into the intestine afford several hundred foci from which digestion takes place, instead of one, as in case of the tablet. The enteric-coating of the preparations is reported by the manufacturers to be composed of a resinous substance soluble in alcohol and alkalies, its solubility being tested in a 2% sodium bicarbonate solution having a pH of 8.4. Sansum ('32) previously showed by x-ray technic that this coating remains intact as long as the pellets stay in the stomach, but that it soon dissolves after reaching the small bowel.

Although the pancreatin employed in the present studies was obtained from two sources, the coated and uncoated (control) preparations for any one experiment were taken from the same manufactured lot and were consequently of the same strength. Standardization tests made on fresh material showed the proteolytic strength of the various preparations to be between 250 and 325% according to the casein assays of the tenth revision of the U.S.P. Although a satisfactory method of assay for lipase has never been devised, attempts were made to determine the relative effectiveness of the coated and uncoated granules on olive oil at varying H-ion concentrations.[1] The titration figures of five assays carried out

[1] Steudel ('33) has stressed the difficulties of assaying lipase and agrees with Willstätter that the only way to estimate the content of this enzyme is to construct a curve of weight and saponifying power for any given preparation, and to utilize its form as a basis of assay.

according to the method of Cherry and Crandall ('32) show such marked variations from experiment to experiment that average values have little meaning. For any one experiment, however, the figures indicate, as shown by a typical experiment summarized below, that for a pH range of 5.6 to 8.4 the two preparations of pancreatin have the same lipolytic strength.

Assay of lipase. Two hundred milligrams of pancreatin and an emulsion containing 7 cc. pure olive oil, 5% gum acacia and a buffered solution, were incubated at 40°C. for 18 hours. The titration figures for the fatty acids liberated are expressed in cubic centimeters 0.2 N NaOH.

	pH 5.6	7.0	7.8	8.4
200 mg. plain granules	18.0	28.3	40.7	35.9
200 mg. coated granules	17.1	27.6	41.3	36.3

In several preliminary experiments increasing amounts of pancreatin were given for the purpose of determining the amount required to complete fat digestion. The addition of 6 or more gm. of pancreatin per 100 gm. of meat frequently produced a severe digestive upset with loss of appetite, marked diarrhea and the passage of large masses of mucus in the stools. The amount of pancreatin given in most experiments reported here was 3 gm. for each 100 gm. of food.

Each experiment was divided into three parts or absorption periods, one in which food alone was given, another in which 3 gm. of uncoated pancreatin (granules or tablets) was given with each 100 gm. of meat, and a third in which an equivalent amount of the enteric-coated pancreatin was given. The order of these parts was changed from time to time. In some experiments the pancreatin was well mixed with the food before feeding, in others it was given after feeding. Each absorption period lasted for six to ten feedings and was demarked by inclusion of charcoal or carmine in the diet.

The animals were trained to defecate on a glazed tile floor so that the feces might be collected easily, quantitatively and immediately following elimination. This procedure had the further advantage of making possible the determination of the elimination time of the feces as the animals were taken

from their individual cages every 3 or 4 hours during the day to permit defecation. Although the stools were usually firm, diarrhea occurred occasionally and the entire absorption period was repeated. Following their collection at the time of elimination, the feces were weighed and preserved with sufficient 20% formalin to give a final formalin concentration to the fecal material of 5%, which is well in excess of the amount necessary to inhibit enzymatic and bacterial action. The pooled samples were kept in air-tight containers in the refrigerator until analyzed.

Since drying of feces results in the loss of ammonia as well as certain fatty acids (Kleiber, Caldwell and Johnson, '36) analyses of fat and nitrogen were usually made on moist feces. In later experiments in which fat alone was determined, the feces were preserved in 95% alcohol and dried over a steam bath. Nitrogen was determined by the usual Kjeldahl method, no correction being made for the nitrogen of the ferment itself. The total ether soluble fats were estimated by the method of Saxon. Although the unsaponifiable fats were estimated in most of the experiments, their value in the present study is questioned and special consideration will not be given them here. All determinations were made in triplicate. The nitrogen and fat of the food were determined by the same methods used in the analysis of the feces.

RESULTS

Lack of space does not permit of the giving of complete results for individual animals. Summaries of fifteen experiments on four dogs, the feces of which were analyzed wet, and of thirteen experiments on six dogs, the feces of which were analyzed dry, are presented in tables 1 and 2, respectively. The figures show changes in the quantity of feces voided and the amount of nitrogen and fat recovered from the diet of lean meat with and without digestive aid (pancreatin).

Weight of feces. The decreased weight of the feces after inclusion of pancreatin in the diet is an outstanding feature of all experiments. Despite the fact that the stools vary

greatly in water content, thus rendering any comparison of weight on wet feces untrustworthy, their reduction in weight with pancreatin was so marked (25 to 60%) in the fifteen experiments in which the feces were analyzed moist (table 1) that mention is made. There was, however, no consistent and significant difference between the weights of wet feces recovered during administration of the coated pancreatin and those recovered during the administration of the uncoated. A more accurate comparison based on the thirteen experiments in which the feces were dried, shows the average decrease in weight after pancreatin was approximately 55%; in several experiments the decrease was well over 60%. In three of nine experiments permitting comparison, the feces collected during inclusion of coated pancreatin weighed slightly more than those collected during administration of the uncoated, in three experiments the reverse was true, and in three the weights were the same. Thus, pancreatin reduces greatly the quantity of feces, but there is no apparent difference between the action of coated and uncoated preparations in this respect.

Elimination time. Another marked change produced by the inclusion of pancreatin in the diet was the definite increase in elimination time, figures for which are not included in the tables. Whereas the elimination time for these dogs when not receiving pancreatin was 12 to 18 hours, during pancreatin feeding it was increased to between 20 and 30 hours. Whether this increased elimination time is due to the reduction of intestinal contents resulting from improved protein digestion, whether the pancreatic enzymes exert a specific inhibitory action on the motility of the alimentary tract, or whether other factors are involved, is not evident from these experiments. Fauley and Ivy ('29) noted that the emptying time of the stomach is decreased more by total pancreatectomy than by ligation of the pancreatic ducts and concluded that hunger is the chief cause of the stomach opening sooner in the absence of pancreatic juice, acidity of the duodenum being less important.

TABLE 1

Efficacy of pancreatin on fat and protein digestion of depancreatized dogs

SERIES	DOG NO.	DIET					FECES								
							Weight of moist feces [1]			Ratio of fecal fat to food fat			Ratio of fecal 'N' to food 'N'		
		Number of feedings	Total meat fed	Total fat fed	Total 'N' fed	Pancreatin added per 100 gm. food	Without pancretin	With plain granules	With coated granules	Without pancretin	With plain granules	With coated granules	Without pancretin	With plain granules	With coated granules
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	%	%	%	%	%	%
1A	1	10	2000	50.0	69.24	3 granules	183	145	117	18.7	32.6	21.8	15.2	11.6	10.8
	2	8	1600	40.0	55.38	3 granules	174	106	100	23.8	28.1	26.2	20.0	12.0	9.4
	3	8	1600	40.0	55.38	3 granules	197	115	107	18.2	29.2	31.8	18.4	12.0	12.4
	4	8	1600	40.0	55.38	3 granules	224	178	176	22.8	33.6	37.2	32.9	13.4	15.3
2A	1	6	1800	57.6	54.33	3 granules	392	190	209	19.5	20.8	21.8	36.0	8.5	10.5
	2	6	1200	38.4	36.22	3 granules	258	192	191	20.4	40.0	35.0	32.4	20.8	21.6
	3	6	1800	57.6	54.33	3 granules	439	162	193	28.6	31.0	22.4	22.3	8.8	8.4
3A	1	9	1800	153.0	57.75	3 granules	540	459	420	27.0	39.0	32.9	27.5	12.2	13.4
	2	8	1600	136.0	51.20	3 granules	528	379	357	29.7	45.8	37.7	31.0	13.8	14.3
	3	8	1600	136.0	51.20	3 granules	535	380	362	25.6	45.2	36.0	29.9	16.0	13.5
	4	8	1600	136.0	51.20	3 granules	459	302	308	43.3	48.7	52.6	27.6	18.5	14.8
4A	1	6	1800	78.3	56.76	3 tablets	566	380	266	31.6	33.8	25.6	27.2	15.1	10.4
	4	6	1800	78.3	56.76	3 tablets	455	228	243	20.4	26.8	23.5	25.0	10.3	9.0
5A	1	7	2100	91.3	67.60	3 tablets	543	332	374	22.0	28.4	31.1	29.4	10.6	15.3
	4	8	1600	69.6	51.39	3 tablets	328	246	223	18.3	22.4	31.4	21.2	11.8	10.8

[1] Nitrogen and fat were determined on wet feces preserved with formaldehyde.

Nitrogen absorption. Following the addition of pancreatin to the diet, fecal nitrogen was diminished by 35 to 65%, the average reduction being approximately 52%. Thus, protein digestion was markedly and uniformly improved by the pancreatin. That there was no significant difference, however, in the effectiveness of the two pancreatin preparations on protein digestion, is indicated by the fact that while the coated reduced fecal nitrogen more (by 2% or more) than did the uncoated in four experiments, the reverse was true in three experiments and the values were approximately the same for the remaining eight.

Fat absorption. The figures indicate that fat digestion is not benefited by the addition of either the coated or uncoated pancreatin, and, as a matter of fact, its addition in the amounts given suggests that inhibition of fat digestion occurred in some experiments. Thus, in fifteen experiments in which the feces were analyzed wet, the average recovery of fat without pancreatin was 24.6% of the ingested fat, as compared with 31.1 and 33.7% for the coated and uncoated pancreatin, respectively. Without pancreatin the fecal fat was definitely less (by 4% or more) in nine experiments, more in two experiments and approximately the same as with pancreatin in the remaining four. Comparing the figures for the coated and uncoated pancreatin, we find the recovered fat was less (by 4% or more) for coated pancreatin in seven experiments, but more in three experiments and the same as the uncoated in five.

While values for experiments in which the feces were dried (table 2) are considerably lower than those obtained on the wet feces, the same general results are noted, namely, that fat elimination is not checked by pancreatin, and that the plain and coated pancreatin give approximately the same results. Three experiments of this series show less fecal fat without pancreatin than with, two show more, and eight have values which are the same as those for pancreatin. Averaging the figures, we find that 10.49% of the ingested fat was recovered without pancreatin, 11.02% with coated and 11.42 with the uncoated.

TABLE 2

Efficacy of pancreatin on fat digestion of depancreatized dogs

SERIES	DOG NO.	DIET					FECES					
							Dry weight			Ratio fecal fat to food fat		
		Number of feedings	Total meat fed	Fat content	Total fat fed	Pancreatin fed per 100 gm. food	Without pancreatin	With coated granules	With plain granules	Without pancreatin	With coated granules	With plain granules
			gm.	%	*gm.*	*gm.*	*gm.*	*gm.*	*gm. N*	%	%	%
1B	5	7	2100	5.6	117.6	3 granules	176.8	76.3	77.0	15.86	21.40	18.70
	6	7	2100	5.6	117.6	3 granules	106.6	60.2	...	12.75	11.48	....
2B	5	8	2400	4.2	100.8	3 granules	215.0	71.0	60.5	13.66	15.31	17.61
	7	8	2400	4.2	100.8	3 granules	195.0	78.6	...	25.47	26.92	....
3B	5	6	2400	5.1	122.4	2½ granules	222.5	67.0	75.3	15.40	12.90	13.75
	7	6	2400	5.1	122.4	2½ granules	148.5	77.7	82.1	8.34	12.36	12.67
	8	6	2400	5.1	122.4	2½ granules	92.0	76.8	72.2	9.54	11.08	10.53
4B	5	6	2400	5.1	122.4	2½ granules	145.0	71.0	65.0	8.88	8.04	7.37
	8	6	2400	5.1	122.4	2½ granules	76.0	51.0	54.0	6.02	5.70	6.22
5B	5	7	2100	5.54	116.3	3 tablets	123.0	62.0	46.0	10.39	8.10	9.78
	8	7	2100	5.54	116.3	3 tablets	58.0	40.5	45.0	7.88	7.83	9.78
	10	7	2100	5.54	116.3	3 tablets	66.0	44.0	41.0	8.96	7.49	8.02
	12	7	2100	5.54	116.3	3 tablets	270.0	...	181.0	47.60	....	44.50

Additional observations. In several preliminary experiments increasing amounts of pancreatin were used to determine the amount necessary to complete the digestion of fat. It was soon learned that regardless of the amount given, fat was not completely digested; in fact, large amounts seemed less effective than small. Protein digestion, however, was improved with larger amounts. Thus, in two experiments on two dogs, the N recovered from the feces was 27.7 and 27.4% of the ingested N. With 3 gm. of pancreatin per 100 gm. meat the percentage of N recovered dropped to 16.6 and 14.2, and with 6 gm. it was 12.8 and 10.4. In a third experiment, in which the increase in pancreatin was in 2 gm. amounts, the N recovered without digestive aid was 29.4% of the ingested N, with 2 gm. pancreatin it was 15.8, with 4 gm. 10.3, and with 6 gm. 9.5. Similar results were reported by Nasset, Pierce and Murlin ('31). That the improvement is not more striking with the larger amounts is probably due to the fact that, although the degree of acceleration of a hydrolytic reaction is proportional to the concentration of the enzyme present, the final amount of products formed is independent of the amount of catalyst. Time and the initial concentration of the substrate are also important factors controlling the amount of food undergoing transformation in the alimentary tract.

Mention should be made of several observations on the passage from the intestine of intact enteric-coated tablets. On one occasion the stool of dog no. 3 and on three occasions the stools of dog no. 8 contained whole tablets. As the passage of the tablets occurred without any accompanying intestinal disturbance, evidenced by the fact that the animals ate well at the time and were free of diarrhea, the occasional failure of the resinous coating to disintegrate is of interest. The manufacturers of the tablets have also noted a variation in the solubility of the enteric coating in different individuals. Their observations with the fluoroscope showed that in most patients the tablet passes through the stomach and disintegrates readily in the small intestine, although in some patients

the same tablet disintegrates in the stomach, and in still others it is carried through the intestinal tract intact (Bibbons, '34).

GENERAL DISCUSSION

That fat digestion is not greatly impaired in dogs deprived of pancreatic juice and given moderate amounts of fat was also reported by Handelsman, Golden and Pratt ('34) on duct-ligated animals. Since in the absence of pancreatic juice as much as 50 gm. of fat may be absorbed daily by a 10 kg. dog, it is apparent that the lipases of gastric and intestinal juices play an important role in the digestion of fat.

An explanation of the failure of pancreatin to improve fat digestion is wanting. That fat absorption may even be made worse by its inclusion in the diet is indicated by the fact that in many experiments more fat was recovered from the feces with pancreatin than without. It is possible that the proteolytic enzyme of the pancreatin inactivated to some degree the gastric and intestinal lipases. Falk ('33) has found that trypsin greatly decreases the effect of lipase on various substrates. Insufficient knowledge of the lipases, however, does not justify further theorizing along this line. Experiments in which only pancreatic lipase is given are now in progress and will be reported later.

That the proteolytic enzyme of the coated pancreatin is no more effective than that of the uncoated is probably explained on the grounds that the presence of food in the stomach offers protection to the enzyme from the gastric juice, and in this respect the in vitro experiments mentioned above differ fundamentally from these. Macleod ('20) states:

> If pepsin is present together with trypsin in a distinctly acid solution, the pepsin seems to destroy the trypsin, unless the mixture contains a considerable quantity of protein, when the tryptic activity may persist even for several hours. A practical conclusion which we may draw from these results is the effect that preparations of trypsin—the so-called, *pancreatin*, for example,—if given with the food, may pass in an active condition into the duodenum, where, in the more favorable environment created by the neutralization of the excess of acid, it will develop its proteolytic power. The therapeutic administration of pancreatin is, therefore, justified.

Inactivation of enzymes by changes in the H-ion concentration and by other enzymes is probably not a simple assault on the enzyme itself, as various authors have pointed out. Thus, Willstätter ('23) found that lipase extracted from gastric mucosa exhibited optimum activity at pH 5.5 to 6.3 and complete inactivation at pH 8.6. After repeated purification with kaolin, the optimum pH was found to be near 8.0, or identical with pancreatic lipase. The enzyme, as secreted in the stomach, is presumably associated with a substance which inhibits its action in a more alkaline medium. According to Willstätter and associates ('22–'24), enzymes are associated with carriers which are chiefly protein in nature, and which have nothing to do with the catalytic effect of the enzyme per se. These workers attribute the inactivation of enzymes by proteases to digestion of the protein carriers, not the enzymes themselves. In pure form many enzymes contain little nitrogen, or else are free of protein (Tauber, '35). Kleiner and Tauber ('34) have recently obtained a trypsin preparation which does not contain protein of the ordinary type, as indicated by the biuret, Millon and heat coagulation tests. Northrop and Kunitz ('34) have also described a highly active crystalline preparation of trypsin, which suffers no loss of activity and no denaturation when a solution is heated in acid to boiling.

In the test tube and under ideal conditions pepsin may inactivate trypsin by digesting its carrier, or by assaulting the free enzyme itself. In the stomach containing food, however, peptic digestion is certainly not carried sufficiently far to bring about irreversible inactivity of trypsin. Hence, trypsin of uncoated pancreatin is as effective as that of the coated, regardless of whether the pancreatin is mixed with the food before ingestion or after. The addition of an enteric coating to pancreatin, therefore, seems unnecessary. In some instances the coating may be definitely disadvantageous, as it occasionally fails to disintegrate in the small intestine and thus prevents liberation of the enzymes.

SUMMARY

The effectiveness of several commercial preparations of plain and coated pancreatin on the digestion of ten totally depancreatized dogs, sustained with insulin, was studied. The addition of 3 gm. of pancreatin per 100 gm. of fresh ground meat 1) reduced the quantity of feces by 30 to 60%, 2) increased the elimination time to approximately normal values, 3) reduced the nitrogen loss of the feces by 30 to 60%, but 4) failed to check the loss of fat in the feces. Larger amounts of pancreatin were more effective than small in the digestion of protein, but were no more effective, possibly less effective, than small in the digestion of fat. There appears to be no uniform significant difference between the action of coated and uncoated pancreatin on digestion of fat and protein.

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ON THE EFFECTIVENESS OF ORALLY ADMINISTERED DIASTASE IN ACHYLIA PANCREATICA (DOG)

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Although there are a few reports in the literature which show that the oral administration of raw pancreas or pancreatin reduces the fat and nitrogen lost in the feces in pancreatic achylia (Ivy, '35), we have been unable to find a worthy report showing that orally administered diastase is effective in reducing the loss of starch. This work was undertaken to obtain controlled evidence on the question, using diastases which are inactivated at a relatively low and high pH.

METHODS

Chemical method for estimating the starch and reducing sugar content of the feces. The starch and reducing sugar content of the feces was determined by a method devised by us. The method is based on the following principles: The feces are dried with alcohol and ether, thoroughly mixed, and an aliquot is suspended in a buffer solution and boiled to render the starch soluble. The mixture is then digested with Takadiastase until all of the starch has been changed to reducing sugars. The mixture is then centrifuged, sugar determinations are made on the supernatant fluid, and the starch content of the feces is calculated.

The feces are collected as soon as possible after passage and macerated with approximately 5 volumes of alcohol.

When convenient the mixture is filtered with suction and washed with additional alcohol. The residue on the paper is washed with ether, placed in a beaker and set aside to dry. This method of drying the feces was used because it is quicker and removes certain of the non-sugar reducing substances. The dried feces are then weighed and two 1-gm. samples are taken for the starch determination. Each sample is placed in a 250-cc. flask containing 90 cc. of pH 4.5 buffer solution, and refluxed for 10 minutes. This insures complete solution of any starch present. The fecal suspension is then cooled. Ten cubic centimeters of an active 2% solution of Takadiastase[1] is added to one of the samples. To the other is added 10 cc. of a 2% solution of Takadiastase which has been previously boiled. The latter sample serves as a control to estimate pre-formed and non-sugar reducing substances. These samples are then incubated at 37° for 2 hours.

At the end of the incubation period the samples are centrifuged and the supernatant fluid analyzed for reducing substances. Although any reducing sugar method may be used for this determination, we have used the hypoiodate titration method as modified by Schmidt, Greengard and Ivy ('34). In this method 5 cc. of the supernatant fluid is added to 10 cc. of N/10 iodine. This mixture is then alkalinized by slowly adding 50 cc. of N/8 NaOH with constant shaking. It is set aside for 15 minutes then acidified with 2 cc. of 10% sulphuric acid and the excess iodine titrated with N/20 sodium thiosulphate.

The reducing substance in the two samples, expressed as maltose, is then calculated according to the following formula:

$$\frac{(10 - \frac{T}{2}) \times 17.15 \times 100}{5} = \text{mg. reducing substance, as maltose, in 1 gm. sample of feces.}$$

T = sodium thiosulphate titration value

The reducing substance value for the control is then subtracted from the reducing substance value of the test determination, leaving the reducing substances, expressed as

[1] Commercial Takadiatase contains a diluent. The product used by us was obtained in pure form from Parke, Davis & Co.

maltose, that have been produced by the digestion of starch. After repeated determinations we have found that 1 gm. of starch treated by this method will yield approximately 1.4 gm. of reducing substance expressed as maltose. The quantity of starch in the 1-gm. sample of feces analyzed is calculated by dividing the maltose value obtained above by 1.4.

The accuracy of the method was tested by analyzing samples of feces from normal dogs and men to which known quantities of starch had been added. The result of one such series of determinations is given in table 1. The maximum error of the

TABLE 1

SAMPLE NUMBER	CALCULATED STARCH CONTENT	DETERMINED STARCH CONTENT	RECOVERED
	%	%	%
1	30	27.4	91.3
2	30	30.4	101.0
3	30	30.0	100.0
4	30	31.8	106.0
5	30	28.7	95.6
6	30	30.5	101.5
7	30	29.4	98.0
8	40	41.1	102.7
9	40	36.5	91.2
10	40	35.3	88.3
		Average	97.6

[1] Numerous series of such tests were made and the results in this table show the maximum differences.

method in our hands is $\pm$ 11%, which is more accurate than other quantitative methods we attempted to employ.

The conversion factor, 1.4, was determined by digesting a sample of cornstarch to reducing sugar according to the method. In other words 1 gm. of the cornstarch we used will yield 1.4 gm. of reducing sugar expressed as maltose. This does not mean that all forms of starch will have this same conversion value. It should be remembered, therefore, that the values obtained by this method are in terms of cornstarch and may not represent the true quantity of other types of starch. The method possesses one other inherent limitation. In addition to digesting the starch to sugar, the Takadiastase

digests small quantities of some other substance, probably protein, to reducing substances. Thus, we have consistently obtained values of from 0.5 to 2.0% 'starch' in samples of feces that have been proven by microscopic examination and the iodine test to be free of starch. Takadiastase is used in the method in spite of this limitation because after repeated tests using different enzyme preparations it proved to be most satisfactory. Since the maximum error of the method is only ±11%, we consider our experimental data on the dogs to be accurate, and greatly within the biological variations.

Physiologic method. Six dogs made totally deficient in external pancreatic secretion by separation of the pancreas from the duodenum were employed. The dogs were then given 1 kg. daily of a diet containing 62% starch (dry weight). The diet consisted of farina cereal, 12%; cod liver oil, 1.6%; lard 1.6%; canned salmon, 9.0% (wet weight); water 75.8%; salt to flavor. The mixture was cooked and prepared in large batches and kept in the ice box. The feces were collected fresh and the 24-hour excretion analyzed for starch. After the dogs were placed on the diet for several days, a control period was started and then the various diastase preparations were given. The feces passed for the first 3 days of any test period were discarded. Four control periods were conducted on most of the dogs.

Potency of enzyme preparations used. A number of diastase-containing enzyme preparations were assayed for potency; some were found inactive or practically so. Only those of strong potency and easily obtained were chosen for our experiments. The potency of the different preparations used are shown in table 2.

Takadiastase (Parke, Davis & Co.) was selected because it is inactivated at a low pH (pH 2.4) and because it is used clinically. Desiccated malt extract was chosen because it is inactivated at a similarly low pH (pH 2.5) and is a relatively inexpensive source. A commercial food preparation (Ovaltine) was chosen for the same reason, since we found it to contain appreciable diastatic power. Pancreatin was tested

because it is used clinically and is inactivated at a higher pH than the plant diastases, and for this reason differences might be found. Enteric coated pancreatin tablets were used to ascertain if the coating increased effectiveness.

The various enzyme preparations were administered in the doses shown in table 3. Half of the dose was mixed with the test meal immediately before feeding and the remainder was given in or with water after the meal had been ingested. The total daily dosage of each preparation expressed in terms of saccharogenic and liquefying power is given in table 2. This method of administration was used because of results obtained in a previous study (Ivy, Schmidt and Beazell, '36).

TABLE 2

PREPARATION	WILLSTATTER UNITS PER GRAM	TOTAL DAILY DOSE IN WILLSTATTER UNITS	GRAMS STARCH HYDROLYZED IN 15 MINUTES AT 37° C. PER GRAM OF PREPARATION	GRAMS STARCH HYDROLYZABLE TO MALTOSE BY TOTAL DAILY DOSE (IN 15 MINUTES)	STARCH LIQUEFYING UNITS (DAVIDSON) PER GRAM	TOTAL DAILY DOSE IN LIQUEFYING UNITS
Pancreatin						
Enteric tablets	7.20	180.0	35	875		
Powdered	6.20	155.0	32	800	55,000	1,375,000
Dry malt extract	1.60	40.0	9	225	3,060	76,500
Takadiastase	0.96	24.0	5.5	137	11,260	281,500
Commercial food preparation	0.56	28.0	3.5	175	2,200	110,000

Greater effectiveness might have been obtained, if further enzyme had been administered with milk, which buffers acid, at 2 and 3 hours after the meal. We did not do this because it would have meant treating the dogs during the control period with milk at those hours.

RESULTS

Prior to the separation of the pancreas from the duodenum the dogs passed no starch in their feces while receiving the high starch diet. After the separation of the pancreas, the dog's feces contained from 18 to 39% starch as may be noted in the columns headed 'first series,' table 3. The dogs divide

TABLE 3

	FIRST SERIES OF TESTS				SECOND SERIES			
	DOG NO.	Days on test	Average starch per 100 gm. feces per day	Per cent decrease in fecal starch	DOG NO.	Days on test	Average starch per 100 gm. feces per day	Per cent decrease in fecal starch
Starch diet alone	1	4	33.4		1	16[1]	33.9	
					2	16	19.1	
Control	3	4	33.4		3	16	29.3	
	4	4	18.0		4	16	19.0	
	5				5	16	19.6	
	6	3	39.2		6			
Average			25.6				24.2	
Starch diet with Takadiastase 25 gm.	1	4	13.7	59.6	1	3	13.9	59.0
	3	4	14.6	56.3				
	4	4	12.8	29.0	4	3	11.5	40.0
	6	4	14.9	61.8				
Average			14.0	51.6			12.7	49.5(?)
Starch diet with malt extract 25 gm.	1	4	16.1	51.8				
	3	4	16.7	50.0				
	4	4	14.3	20.5				
	6	4	16.5	57.9				
Average			15.9	45.0				
Starch diet with commercial food preparation 50 gm.	1	4	20.2	39.5	1	14[2]	17.4	48.7
	2				2	14	7.6	60.0
	3	4	19.9	40.5	3	14	17.1	41.7
	4	4	15.5	14.0	4	14	17.7	7.0
	5				5	14	10.5	46.5
	6	4	20.2	48.5	6			
Average			18.9	35.6			14.0	40.7
Starch diet with Pancreatin 25 gm.	1	4	25.2	24.5	1	5	26.4	22.0
	3	4	16.7	50.0				
	4	4	14.8	18.0	4	5	12.5	34.0
	6	4	12.5	68.1				
Average			14.7	40.1			19.4	28.0(?)
Starch diet with Enteric Pancreatin tablets 25 gm.	1	4	14.6	56.5				
	3	4	14.4	57.0				
	4	4	13.7	23.7				
	6	4	14.3	63.5				
Average			14.0	50.1				

[1] Average of one 8-day and two 4-day control periods.
[2] Average of two 7-day test periods.

themselves into two groups in regard to a high (dogs 1, 3, 6) and low (dogs 2, 4, 5) loss of starch. It is to be noted that each dog from one control period to another was remarkably constant in regard to the percentage of starch lost in the feces. Table 4 shows the results of a typical test on dog 1, and is given to show the variation from day to day. To conserve space, three of the control periods are averaged in the column headed 'second series' in table 3. The second series of tests with diastase (Takadiastase, pancreatin and commercial food preparation) yielded results that as a whole check as well as could be expected, except in the case of dog 4.

TABLE 4

Typical series of determinations showing daily variation in starch excretion. Dog no. 1

STARCH DIET ALONE		STARCH DIET PLUS COMMERCIAL FOOD PREPARATION	
Day of diet	Starch per 100 gm. feces	Day of diet	Starch per 100 gm. feces
1	34.2	1	14.3
2	19.4	2	18.1
3	29.0	3	18.3
4	33.7	4	33.3
5	51.0	5	19.5
6	42.0	6	19.4
7	28.0	7	19.0
Average	33.9	Average	20.1

The results in table 3 are expressed on a percentage basis because, since the stools are frequently mushy, it was technically impossible to collect all of the feces each day. However, calculations were made of the percentage of ingested starch actually lost, and the results, as might be expected, were roughly proportionate to the percentage changes. Only the percentage figures are given in the tables because we are certain of their accuracy within the limits of our method. The effect of the addition of enzymes is very definite. Averaging the results of series one and two on Takadiastase the percentage reduction in fecal starch is 50; the percentage reduction with malt extract is 45 and with the commercial food

preparation is 38.5. The average percentage reduction with pancreatin powder is 36.1 and with the enteric coated pancreatin tablets 50.1.

DISCUSSION

The oral effectiveness of diastase in reducing the starch loss in the feces of dogs with pancreatic achylia is quite obvious from the results. This might be expected since we (Ivy, Schmidt and Beazell, '36) have shown that when malt amylase, or even ptyalin, is administered with a meal a considerable portion of it passes into the intestine before the pH of the gastric contents is lowered to the point of inactivation of the enzyme.

Although the averages indicate that Takadiastase may be slightly superior to malt diastase, in view of the fact that fewer saccharogenic units of the former were administered, we doubt that our data permit such a deduction. Further, the greater liquefying power of Takadiastase as compared with malt diastase must be considered. However, we do believe that malt diastase (45%) is more effective than pancreatin powder (36.1%) on the basis of the difference (8.9%) in the averages and the large differences in the units of enzyme (800 units of pancreatin and 225 of malt extract) administered. This is not surprising in view of the fact that pancreatic diastase is inactivated at a much higher pH relatively than malt diastase. The data show very clearly that the enteric coating increases the effectiveness of pancreatin.

The differences between the dogs, of course, is to be expected, because it is well known that some dogs withstand pancreatic achylia much better than others.

Attention should be directed to the relatively large doses we employed to obtain the above results and the fact that in no instance was the starch completely eliminated from the feces. On a lower starch diet and in dogs whose gastric acidity does not rise rapidly post-cibum, it is probable that smaller doses would completely eliminate starch from the

feces. Also, amylorrhoea would probably not be as marked in the human in pancreatic achylia as in the dog because the dog produces no ptyalin.

SUMMARY AND CONCLUSIONS

Absolute pancreatic achylia was produced by separating the pancreas from the duodenum in six dogs, which were fed a high starch (62%) diet. Prior to the production of the achylia the dogs did not show amylorrhoea while on the diet. After the production of the achylia the starch in the feces varied from 18 to 39% in the different dogs. The administration of diastatic enzymes in relatively large amounts very definitely reduced the starch loss in each dog, proving the effectiveness of oral diastatic therapy in pancreatic deficiency. The vegetable diastases were more efficient than pancreatic amylase (pancreatin) which is most probably due to the latter being more readily inactivated by the gastric acidity than the former. Enteric coating of the pancreatic amylase (pancreatin) rendered it as effective as the vegetable diastases; but the enteric coated pancreatin was not as effective as the vegetable diastases on the basis of the enzymic units administered.

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THE BILATERAL SYMMETRY OF SKIN TEMPERATURE

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ONE FIGURE

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Previous investigations of the physiological state of the schizophrenic patient have shown an abnormal variability of various processes and, in certain respects, a displacement of mean functional levels (Hoskins and Jellinek, '33). In Cannon's terminology, the schizophrenic patient seems to show abnormal homeostasis. Such adaptative inefficiency lends itself readily to quantitative analysis from the viewpoint of the effects of varying temperature, humidity and air velocity upon the heat regulating mechanisms.

A dysfunction of heat regulation in this psychosis is suggested by several studies. Gottlieb and Linder ('35) have found that on exposure to changes in the environmental temperature from 73°F. to 93°F. patients exhibit significantly greater increases than do those of normal individuals. Carmichael and Linder ('34) have noted that there is a higher correlation between oral and rectal temperatures in schizophrenic subjects than in normal controls, thereby implying in the former group a lessened adaptability to environmental or internal variations. Hoskins and Walsh ('32) have reported a decreased oxygen consumption rate, i.e., a diminished heat production in this psychosis. Finkelman and Stephens ('36) have observed in a study of the response to cold in schizophrenic and normal subjects that in the former group the heat

production was lower, that it was not maintained as long, and that there was no reactive hyperemia.

The experiments to be described in this and subsequent papers have been studies of the skin temperatures, body temperatures and oxygen consumption rates of normal and schizophrenic individuals under varying environmental conditions. Many temperature studies of normal individuals have been reported. Since few have been performed with a sufficient number of subjects or under adequately controlled environmental conditions to give statistically reliable values, it has been necessary to establish our own normal standards.

The environmental control was secured by the construction of a psychrometric laboratory provided through the generosity of the Rockefeller Foundation. In this laboratory the equipment was designed to produce on any day in the year dry bulb temperatures varying from 10°C. to 50°C. with relative humidities varying independently from 20% to 90% throughout the temperature range. The air velocity could be held constant or could be varied through a wide range.

For the study of temperatures copper-constantin thermocouples were used, one for skin, two for oral and two for rectal determinations. The various thermocouples were connected by a multiple switch so that all could be read in rapid succession through the medium of a galvonometer calibrated to be read in temperatures, directly. The oxygen consumption rate was determined by means of a Benedict-Roth metabolism instrument. The air velocity was measured on several occasions each day by means of a kata-thermometer (Angus, Hill and Soper, '30). In order to ensure as nearly complete access as possible of all environmental factors to the entire surface of the body, the beds were constructed of coarsely woven cane with wide interstices. No mattresses, blankets or sheets were used. For the convenience of the subjects sponge rubber mats were placed under the head and heels.

Forty subjects were used for the experiments, twenty being healthy men and twenty men suffering from schizophrenia, but otherwise without disease. The control and psychotic subjects were approximately matched for height and weight.

They lived in adjacent rooms, took approximately the same amount of exercise and ate the same type of food. Thus for each group the extra-experimental conditions were quite comparable.

Each morning at 9.00 A.M. a patient and his matched normal control were conducted from their beds to the laboratory in a fasting state, clad in bathrobes and slippers. On their arrival they disrobed immediately and lay upon the beds. As a further precaution to ensure similarity of conditions between the two groups successive pairs of subjects occupied alternate beds in the experimental room. Immediately after recumbency and at every half hour thereafter from 9.00 A.M. to 12.00 M. measurements were taken of the skin, oral and rectal temperatures and the oxygen consumption rates. Thus, seven sets of readings were taken each morning, each of these repetitions being designated as a 'period.' The skin temperature was measured at nineteen points, each stamped by an inked circle 0.5 inch in diameter in order to be sure that the same spot was measured on subsequent examinations, as slight variations in localization have been found to show marked differences in temperature (Freeman and Linder, '34). The areas measured are shown in figure 1.

The effects of various environmental conditions were studied. The number of measurements of skin temperature obtained in these studies has been so great that the desirability of utilizing only those readings pertaining to one side of the body was considered. This reduction of the data would be permissible only if a rigid analysis revealed a high degree of consistency between the temperatures of the two sides. This paper presents the results of such an analysis.

A similarity of the skin temperatures of symmetrically located areas of the body has been noted by several investigators (Cobet, '26; Talbot, '31). However, the actual degree of similarity has not been determined under controlled environmental conditions and with rigid attention to quantification. The results of such a study should prove interesting not only from the viewpoint of human physiology but also from that of pathology.

In the present investigation, after ten pairs of subjects had been studied, the experiment was interrupted for 6 weeks and then resumed. The average ward temperature, i.e., pre-test environment to which the second ten pairs were exposed, was significantly higher than that for the first ten pairs (21.1°C. versus 18.9°C.). The average air velocity in the experimental

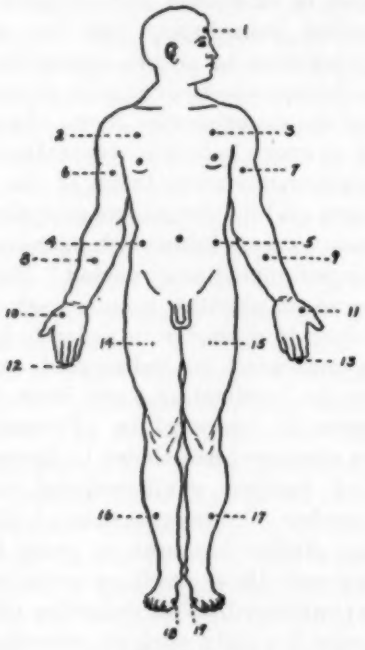

Figure 1

room was also significantly greater during the second series (40.7 feet per minute versus 22.5 feet per minute). In both series, however, the dry bulb temperature was maintained at 24°C. and the relative humidity at 20%. In view of the possible influence of these changes on skin temperature the data have been analyzed separately for each group of ten and will be so presented.

The average differences between the right and left sides for the nine locations are shown in table 1.[1] Although these differences are rather small, two-thirds of them are statistically significant, i.e., are beyond the probability of chance occurrence. They are indicative of the fact that in some areas the right side is consistently warmer and in others the left. The consistency of the signs of the differences of the two groups of controls as well as patients lends additional weight to their significance. Over the trunk, seven out of the eight differences show the left side to have the higher temperature; on the arm and hand the right side is higher in all of the sixteen instances. It would seem then, that in both the normal control and schizophrenic subjects there is a systematic variation in the average temperature difference between the two sides over the various portions of the body surface. The trunk is warmer on the left side, the arm and hand on the right, the thigh on the left and the lower leg and foot on the right. It is of interest that in a small series of normal subjects Heiser and Cohen ('33) found, from a comparison of the wrist and ankle skin temperatures that those on the right were signficantly higher than those on the left side. The explanation for this systematic variation is uncertain. It may possibly be ascribed to anatomic differences over the trunk and to the relative dominance of the right limbs. It cannot be ascribed definitely to 'handedness' for in the twenty patients only one was left handed and yet eight had lower temperatures on the right hand. Notwithstanding the significance of these differences, their relatively small magnitudes suggest a rather high degree of similarity between the temperatures of the two sides.

In addition to this description of mean differences knowledge is required of the variation that takes place between sides from individual to individual, from period to period, and of the net variation within the individual.

Values descriptive of the variation of the differences between sides from individual to individual are given in table 2

[1] Each of these average differences is based on seventy separate differences.

TABLE 1

Average differences in skin temperature (°C.) between the right and left sides in nine symmetrically located areas for seven sets of readings in twenty normal controls and twenty schizophrenic subjects (the average temperature of the left side is subtracted from that of the right side

	CHEST		ABDOMEN		UPPER ARMS		LOWER ARMS		THUMBS		FINGERS		THIGHS		LOWER LEGS		FIRST TOES	
	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.
1st 10	—0.17[1]	—0.04	—0.03	—0.03	0.11[1]	0.03	0.10[1]	0.08[1]	0.05	0.12[1]	0.07	0.34[1]	—0.09[1]	—0.14[1]	—0.10[1]	0.05[1]	0.09[1]	0.00
2nd 10	—0.09[1]	—0.09[1]	—0.03	—0.05	0.09[1]	0.11[1]	0.12[1]	0.16[1]	0.11	0.47[1]	0.19[1]	0.22[1]	—0.26[1]	—0.47[1]	0.29[1]	0.06[1]	0.02	0.02

[1] Denotes magnitudes that are statistically significant. Significance has been determined by means of Fisher's 't' test ('34) using the values in table 4 as a basis of reference.

TABLE 2

Inter-individual variation (standard deviation[1]) of differences in skin temperature (°C.) between right and left sides for nine symmetrically located areas over seven sets of readings in twenty normal controls and twenty schizophrenic subjects

	CHEST		ABDOMEN		UPPER ARMS		LOWER ARMS		THUMBS		FINGERS		THIGHS		LOWER LEGS		FIRST TOES	
	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.
1st 10	0.39	0.31	0.52	0.43	0.78	0.55	0.64	0.56	0.73	0.42	0.55	0.44	0.31	0.34	0.40	0.35	0.82	0.46
2nd 10	0.60	0.45	1.00	0.36	0.85	0.60	0.64	0.67	0.68	1.12	0.72	0.91	0.43	0.50	0.65	0.42	0.84	0.44

All values are statistically significant. Significance has been established by Fisher's 'z' test, using the values of table 4 as a basis of reference.

[1] These standard deviations pertain to the variation existing among the mean temperature differences for ten individuals. They are derived from the 'Individual-Side Interaction Variance' isolated by an analysis of variance for three criteria of classification.

for each of the nine areas. There are several interesting items in this table. First, all the values are statistically highly significant. This implies that the individuals are definitely different from each other with respect to the magnitude of the difference between the two sides for any of the nine areas studied. It will be noted that the values of the second group are higher than those of the first in all but three instances. That is, the differences between individuals are exaggerated by the introduction of some factor, such as, possibly, increased air velocity. Second, the magnitude of the variation shows no systematic trend over the nine areas studied. Thus, it would seem that the inter-individual variation of the differences between right and left sides is no greater for the extremities than for the trunk. This is somewhat surprising in view of the observed fact that individuals differ more from each other in the temperature of their extremities than in the temperature of their trunks. Third, so far as the normal control and schizophrenic subjects are concerned, the psychotic individuals have larger values in thirteen of the eighteen instances. This implies that the schizophrenic group is more heterogeneous with respect to the differences between the right and left sides than is the group of normal controls.

Values in table 3 pertain to the variation of the difference between sides as displayed from period to period. These values are all quite small and thus point to a high degree of consistency of the difference between sides from period to period. Of the thirty-six values, but four are sufficiently large to have satistical significance. While the magnitude of the difference between the temperatures of the two sides may be a particular function of the individual, it is independent of the period during which the determination was made. The values in the second group are not appreciably larger than those in the first, implying that the average difference did not fluctuate any more over the seven periods of this latter series in spite of the increased air velocity. Again the extremities do not seem to be affected to any greater extent in

this regard than are any other areas. In this instance no differences were observed between the patients and the normal controls.

The two sources of variability already discussed dealt with the aggregates of seven periods for each of ten individuals (table 2); and with aggregates of ten individuals for each of seven periods (table 3), thus furnishing data as to the variation of the difference between sides from individual to individual and from period to period. It is desirable, however, to obtain an expression of the average variability within the individual for any given period. In order to arrive at this expression the following procedure is carried out: The intra-individual variation of the difference between the two sides is determined, this quantity revealing the fluctuation of the magnitude of the difference over the seven periods within the individual; then the period-to-period variation of the group (table 3) is subtracted. The resulting values are given in table 4. The small magnitude of these instantaneous measures of variability demonstrate the high degree of similarity between temperatures of symmetrically placed areas. The fact that the values in table 2 are significantly greater than these values has already given rise to the statement that the individual is more consistent with himself than with others. The values for the thumb and finger are somewhat greater than those for other points. This implies that for the individual there is less symmetry of temperatures for these two areas. The second group has higher values with but two exceptions, implying that the intra-individual consistency has been reduced by some such factor as air velocity. Finally the values for patients are somewhat higher than those for the normal controls. There is a suggestion of less consistency between the temperature of symmetrically located areas of the body surface in schizophrenics than in normal control subjects.

TABLE 3

Inter-period variation (standard deviation)[2] of differences in skin temperature (°C.) between right and left sides for nine symmetrically located areas in twenty normal controls and twenty schizophrenic subjects

	CHEST		ABDOMEN		UPPER ARMS		LOWER ARMS		THUMBS		FINGERS		THIGHS		LOWER LEGS		FIRST TOES	
	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.
1st 10	0.10	0.07	0.14	0.10	0.32[1]	0.13	0.26	0.15	0.29	0.07	0.35	0.12	0.11	0.09	0.14	0.22[1]	0.15	0.17
2nd 10	0.30	0.19	0.18	0.15	0.17	0.21	0.22	0.19	0.22	0.24	0.25	0.62	0.08	0.10	0.12	0.12	0.09	0.25[1]

[1] Denotes magnitudes that are statistically significant, as determined from the values in table 4 and the 'z' test.

[2] These standard deviations pertain to the variation existing among the mean temperature differences for seven periods. They are derived from the so-called 'Period-Side Interaction Variance' isolated by an analysis of variance for three criteria.

TABLE 4

Net intra-individual variation (standard deviation)[1] of differences in skin temperature (°C.) between right and left sides for nine symmetrically located areas in twenty normal controls and twenty schizophrenic subjects

	CHEST		ABDOMEN		UPPER ARMS		LOWER ARMS		THUMBS		FINGERS		THIGHS		LOWER LEGS		FIRST TOES	
	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.	Pts.	N.C.
1st 10	0.09	0.09	0.10	0.09	0.16	0.11	0.15	0.13	0.24	0.25	0.33	0.30	0.10	0.08	0.11	0.11	0.18	0.07
2nd 10	0.21	0.14	0.15	0.11	0.19	0.23	0.21	0.21	0.26	0.24	0.38	0.43	0.13	0.12	0.15	0.17	0.15	0.12

[1] These standard deviations pertain to the variation existing within the individual after the period to period variation has been removed. They are derived from the so-called 'Period-Side-Individual Interaction Variance' isolated by a three-way analysis of variance.

SUMMARY

As a part of a biological analysis of adaptative functions in schizophrenia, a study was made of the differences in skin temperature between the right and left sides in nine symmetrically located areas on the body surface of twenty normal and twenty schizophrenic individuals. Seven readings were made on each point at 30-minute intervals with the environmental temperature held at 24°C. and the relative humidity at 20%. It was found that:

1. In both normal and psychotic individuals the trunk on the left and the extremities on the right have the higher skin temperatures.

2. Individuals differ significantly from each other relative to the magnitude of the differences between the temperatures of symmetrically located areas. In this respect, however, schizophrenics differ from each other more than do normal individuals.

3. From period to period the consistency of the magnitudes of the differences between the temperatures of the two sides is very high despite the fact that the levels of temperature may be varying.

4. The variation of the magnitude of the differences between the two sides within the individual for any particular period is very small. In this respect also, the schizophrenic patient shows less consistency between the temperatures of symmetrically located areas than does the normal subject.

5. On the whole it may be safely concluded that the degree of bilateral symmetry of skin temperatures warrants the limitation of the analysis of such data to one side of the body.

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AN IMPROVED TECHNIC FOR METABOLISM STUDIES IN PRE-SCHOOL CHILDREN WITH A STATISTICAL DETERMINATION OF ITS RELIABILITY[1]

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ONE FIGURE

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To obtain more information concerning the factors which influence the nitrogen, calorie and mineral utilization of pre-school children, the investigators in this laboratory started a series of metabolism studies in 1928. Since the quantity of protein required for optimum nutrition is one of the major diet problems, they began the study from that angle. After some preliminary balance studies, they conducted two long-time experiments on pre-school children. There were several purposes for these investigations; first, to find the variations in the nitrogen, calorie and mineral utilization of pre-school children who received a constant diet containing 3 gm. of protein per kilogram of body weight; second, to discover the changes in their metabolism when the protein content of their diet was increased to 4 gm. per kilogram; and third, to find the interrelationships between the utilizations of the various substances.

[1] Published from Michigan State College Agricultural Experiment Station as paper 263, new series.

TECHNIC FOR METABOLISM STUDY

Since the technic used in any balance study largely determines the value of the results, the investigators planned the procedure and controlled the conditions very carefully. The methods used varied somewhat from those of other investigators and they may suggest some improvements in technic. Thus, it seems necessary to present the procedure in some detail before reporting the results.

Although it is impossible to control all physiological differences in individuals, the subjects are more likely to be in somewhat the same condition if they are approximately the same age and if they have lived under similar conditions for a period of time. The two boys, who served as subjects in the first experiment in this study, were each 4½ years of age and had lived practically all of their lives in an orphanage where the food and general habits of the children were carefully controlled. In the second experiment, the two boys were 4 and 4½ years and the two girls were each 3 years of age. These four children had all lived in boarding homes under normal conditions. Physical examination showed that all of the children were in good condition. Their weights, as shown in table 1, were similar to the weight of an average child of the same height and age (Woodbury, '23). The table also indicates that, with the exception of E, they all gained both in height and weight during the time of the experiment. E's failure to gain may be explained by the fact that at the beginning of the medium protein diet he contracted a throat infection and was quite ill for several days. Although he received the medium protein diet for 13 days at the end of the first experimental period, it did not seem wise to include any of those results, but only those from the high protein diet. Thus, his weight record is much shorter than that of the other children. All of the children represented average, normal but not superior children.

It is also impossible to control entirely the psychological effects which a balance study will have on the individual child. During the time of the study the children lived in an apart-

ment in the Home Economics building and were under constant supervision. They followed a definite routine as to play, rest, eating, toilet habits, etc. The children in the first experiment attended the college nursery school, but, on account of an epidemic of colds which occurred, those on the second experiment played by themselves. They all seemed happy and apparently did not miss their former associates.

TABLE 1

Physical measurements of children at beginning and end of experiment

SUBJECT	EXPERIMENT	AGE	HEIGHT	WEIGHT	VARIATION FROM AVERAGE WEIGHT
		months	*cm.*	*kg.*	%
D	I	57	108.4	17.2	—2.6
		59	109.7	17.5	—3.1
B	I	55	109.7	18.6	+2.5
		57	111.0	19.1	+2.4
J	II	38	95.3	13.6	—4.3
		40	96.5	13.9	—4.8
C	II	37	98.6	14.5	—2.0
		39	99.1	14.8	—1.3
V	II	49	102.9	16.9	+5.5
		51	103.9	17.1	+3.2
E	II	57	110.0	18.3	+0.6
		58	110.0	18.3	+0.6

The time of the study was sufficiently long to indicate the general trends in the metabolism of these children and also to show 3-day period variations. In the first experiment, they received a diet containing 3 gm. of protein per kilogram of body weight for a preliminary period of 12 and a collection period of 21 days and a diet containing 4 gm. of protein per kilogram for the following 15 days. In the second experiment, the 3-gm. protein diet continued for 10 preliminary and 21 collection days and the 4-gm. protein diet lasted for the next 24 days.

The plan of the diets removed a number of technical errors which may occur in any balance study. The results for individual children were comparable because every child received an amount of each food proportional to his body weight and thus, the same number of calories and grams of each mineral constituent per kilogram. For example, all of the children did not have the same total amount of milk, egg or other foods, but they received practically the same quantity per kilogram. If the children had been undernourished or obese, the theoretical weight would probably have been a more ideal measurement upon which to base the food needs, but the theoretical and actual weights of these children were practically the same. Since Daniels and her associates ('35) found more comparable results for nitrogen retention based on creatinine excretion, which they believe measures muscle need, it may be that calculations should have been based on this measurement. Creatinine excretion, however, would not indicate mineral needs.

The differences in the results on the medium and high protein diets would probably be due to the change in protein content because the other factors in the two diets remained fairly constant. Table 2 gives the four diets in grams of food for a child weighing 15 kg. In the first experiment, the medium and high protein diets contained the same foods with the exception of skimmed milk. Although the grams of each food were not always identical, the variations were slight, except that additional milk, ground beef and egg raised the protein content. These changes increased the percentage of animal protein from 74.2 to 82.7 and the percentage of total calories from protein sources from 14.1 to 18.5. The distribution of calories from fat and carbohydrate were quite similar, 35.0 and 50.9% in the first diet and 33.7 and 47.8% in the second. The alterations in the quantities of fruit and vegetable gave 12.6 extra gm. to the second diet, which did not radically change the bulk content. Since the mineral constituents did vary to some extent, the acid-base relationship of the diets, calculated according to the method of Salter and

his associates ('31), changed slightly. The excess of base over acid in the medium protein diet was 8.4 cc. of N solution greater than that on the high protein diet. In the second experiment, the diets were identical, except for the increase in protein content by means of egg white and gelatin, which

TABLE 2

Diet calculations for a child weighing 15 kg.

FOOD	FIRST EXPERIMENT		SECOND EXPERIMENT	
	Medium protein	High protein	Medium protein	High protein
	gm.	gm.	gm.	gm.
Milk	607.7	358.7	647.4	647.4
Milk (skimmed)	....	358.7	....	....
Ground beef	38.0	76.0	32.4	32.4
Egg (whole)	38.0	76.0	32.4	32.4
Egg (white)	....	....	....	64.7
Gelatin	....	....	....	8.1
Farina	15.2	15.2	....	....
Ralston's	....	....	16.2	16.2
Bread	60.8	50.6	48.6	48.6
Potato	53.2	42.2	64.7	64.7
Carrots	60.8	59.1	....	....
String beans	....	....	80.9	80.9
Tomato	76.0	84.4	80.9	80.9
Lettuce	11.8	11.8	....	....
Celery	....	....	16.2	16.2
Applesauce	76.0	76.0	121.4	121.4
Prunes	76.0	76.0	....	....
Peaches	....	....	121.4	121.4
Orange juice	151.9	168.8	161.8	161.8
Butter	15.2	16.9	16.2	8.1
Sugar	15.2	8.4	16.2	16.2
Cod liver oil	3.8	3.8	....	....
Haliver oil (250D)	....	....	1 pearl	1 pearl
Total fruit and vegetable	505.7	518.3	647.3	647.3
Protein	45.0	61.1	45.0	60.3
Animal source (%)	74.2	82.7	73.0	79.8
Calories	1282.0	1319.0	1351.0	1352.0
Protein (%)	14.1	18.5	13.3	17.8
Fat (%)	35.0	33.7	31.9	27.4
Carbohydrate (%)	50.9	47.8	54.8	54.8
Calcium	0.912	1.081	0.965	0.973
Phosphorus	1.032	1.282	1.031	1.039
Excess base over acid (cc. N.)	41.2	32.8	32.4	28.3

contained practically nothing but protein, and the omission of an equivalent number of calories from butter, which kept the calorie content of the diets at the same level. Since the extra protein was from animal source, the percentage of animal protein increased from 73.0 to 79.8. The distribution of calories from protein, fat and carbohydrate was 13.3, 31.9 and 54.8% on the first diet and 17.8, 27.4 and 54.8% on the second. Since the mineral content was practically identical, the acid-base relationship changed only 4.1 cc. N solution.

Although the second experiment was slightly more constant than the first, the results would certainly be comparable. There were a few differences in the foods served. In the second experiment, the children preferred Ralston's, string beans and celery to farina, carrots and lettuce. On account of the organic acids formed, peaches replaced prunes and because it was easier to feed quantitatively, haliver oil 250D took the place of cod liver oil.[2] Although the grams of the same foods were not identical in the two experiments, the organic and inorganic constituents were quite similar. In both experiments, the children had a measured quantity of distilled water for drinking.

The preparation and feeding of the food eliminated many of the possible errors. To reduce the probable variations in the composition of the same food, a quantity of canned and dried foods sufficient to last for the entire experiment and enough perishable foods to last for a 3-day period were purchased at one time. Great care was observed in preparing duplicate samples of food for analysis which would be identical with the portions fed to the children. The entire quantity of each food required for a 3-day period was made into a homogeneous mixture. The fat and gristle was removed from meat and the lean portions ground, eggs were beaten, potatoes were cooked and mashed and vegetables and fruits were pureéd. All samples of one food were weighed at the same

[2] The haliver oil was supplied by Parke, Davis and Company, Detroit, Michigan, and the cod liver oil was obtained from E. L. Patch and Company, Boston, Massachusetts.

time in order to avoid changes due to water loss. Losses due to transferring food were avoided by weighing the portions for the children into the same utensils in which they were later cooked and served and by rinsing the samples for analysis into large enamel bowls with distilled water. In order to eliminate spoilage, the egg and meat dishes for the children were heated on the first day and all of the food was stored in an ice box at a low temperature. The samples for analysis were partially dried on a steam bath and then left in a constant temperature oven at 60°C. until they no longer lost weight. They were then ground several times and put through a fine-meshed sieve, the hard portions being mortared before the final sifting, when they were stored for analysis.

The food consumption was as nearly quantitative as possible. The children ate all of their meals under the constant supervision of a tactful helper who taught them to be very careful not to spill their food. She scraped the dishes carefully, wiped them out with a small piece of bread, which the children ate, and finally rinsed them with part of the child's allotted distilled water which she added to another food that the child had not eaten.

The collection and preservation of urine and feces samples were as accurate as possible. To prevent loss of urine samples, the younger children followed a definite schedule as to hours of collection. If, however, an accident occurred, the complete 24-hour sample was discarded and the results for the other 2 days in the period averaged. To lessen decomposition, samples were kept in an ice box at all times until they were complete. After the sample was measured and the specific gravity taken, it was made up to a definite volume with redistilled water. The nitrogenous constituents were determined daily and an aliquot portion of each day's sample was combined into a 3-day composite for mineral determinations. These samples were dried on a steam bath, ashed at a low temperature in platinum dishes, dissolved in 5 to 10% HCl, made up to 100 cc. and saved for analyses. In the first experiment, feces were collected in daily samples, charcoal

being used as a marker one day and carmine the next. A portion of the moist feces was partially digested into 20% H_2SO_4 and Kjeldahl determinations made on this sample. The remainder of the feces was dried. In the second experiment, feces were marked with carmine, collected in 3-day periods, prepared in the same manner as the food and stored until used.

Before each chemical determination, care was taken to obtain a homogeneous sample having the same moisture content as the original dried food. In order to do this the ground food was sifted, thoroughly mixed and a portion of it dried overnight in a constant temperature oven at 60°C. From this sample, triplicate portions were weighed for the estimation of both calories and nitrogen. Calories were determined in an oxy-calorimeter as described by Benedict and Fox ('25 a, '25 b) and nitrogen was estimated by the official Kjeldahl method (Association of Official Agricultural Chemists, '25). For minerals, duplicate samples varying in weight from 15 to 20 gm. were ashed in platinum dishes at a temperature below red heat, dissolved in hydrochloric acid and made up to 100 cc. Calcium was estimated according to the method described by Kramer and Howland ('26) except that the precipitated calcium oxalate was filtered through a porous bottom crucible rather than filter paper. Phosphorus was determined by the uranium titration as outlined by Peters and Van Slyke ('32). In a few cases in which the results did not check within 2%, a third ashing was made and all three results averaged.

STATISTICAL EVALUATION OF THE CONSTANCY OF THE DIET COMPOSITION

Diet calculations from standard tables of food composition give only approximate values, since the composition of individual food varies with a number of factors, such as variety of the product, degree of freshness and the type of soil, climatic conditions and season of the year in which it was grown. Therefore, it is necessary to analyze the food used in order to obtain the actual composition.

There are variations in the composition of identical diets even when the experimental conditions are carefully controlled. Porter-Levin ('33) found that the calcium and phosphorus intakes of her subjects, who received the same diet during a number of periods, varied within fairly small limits. The coefficients of variation were between 2 and 3. Bassett and Van Alstine ('35) obtained similar coefficients for the nitrogen content of identical diets, but higher values, from

TABLE 3

Range and variability in composition of identical diets

ELEMENT	EXPERIMENT	PROTEIN PER KILOGRAM IN DIET	NUMBER OF SAMPLES	COMPOSITION		STANDARD DEVIATION	COEFFICIENT OF VARIATION
				Range	Mean		
		gm.					%
Calories	I	3	18	1444–1558	1497± 4.7	29	2.0
		4	8	1543–1677	1596±10.2	43	2.7
	II	3	16	1132–1219	1171± 3.9	23	2.0
		4	16	1128–1199	1164± 3.8	23	1.9
Nitrogen (gm.)	I	3	18	8.40– 9.19	8.77±0.04	0.24	2.7
		4	10	11.18–12.15	11.85±0.06	0.27	2.3
	II	3	16	5.62– 6.28	6.01±0.03	0.18	3.0
		4	16	7.93– 8.37	8.19±0.02	0.13	1.6
Calcium (mg.)	I	3	18	987–1076	1032±3.8	24	2.3
		4	10	1173–1233	1200±4.0	19	1.6
	II	3	16	716– 793	755±3.9	23	3.1
		4	16	736– 831	776±4.1	24	3.1
Phosphorus (mg.)	I	3	18	1097–1194	1143±4.4	28	2.4
		4	10	1405–1507	1456±8.2	38	2.6
	II	3	16	825– 895	863±3.8	22	2.6
		4	16	833– 915	874±3.2	19	2.2

3.0 to 8.8, for calcium and phosphorus. In the present study the calorie, nitrogen, calcium and phosphorus analyses of identical diets and of duplicate diets collected from the same food on the same day give some further information concerning variability in composition.

Table 3 presents the range in the calorie, nitrogen, calcium and phosphorus values which were obtained by the analyses of from eight to eighteen identical samples of the four different diets. Since Dunn ('29) has shown that statistical

methods may be applied to a small series of analyses, the table also gives the mean with its probable error, the standard deviation and the coefficient of variation. These constants were calculated according to the following formulae:

$$M = \frac{\Sigma(x)}{n}$$

$$\text{P.E. of } M = 0.6745\left(\frac{\sigma}{\sqrt{n}}\right)$$

$$\text{S.D. or } \sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

$$\text{C.V.} = 100\left(\frac{\sigma}{m}\right)$$

The coefficients of variation, which represent the relative extent to which the standard deviations vary from the means, were quite constant for all constituents analyzed in each series of determinations. These values, which ranged from 1.6 to 3.1, showed that although the identical diets did vary somewhat in their composition, the quantity of the different elements varied in about the same degree. Nitrogen figures displayed variations similar to those which Bassett and Van Alstine ('35) reported. The values for calcium and phosphorus, on the other hand, did not show as large variations as these authors found, but fluctuated in about the same manner as the figures which Porter-Levin ('33) gave.

There are several factors which may have caused these variations in composition. In the first place, there were the unavoidable errors in chemical technic. These errors may have produced a large proportion of the discrepancies, because Bassett and Van Alstine ('35) found that the coefficients of variation in the results of a number of consecutive analyses of a single specimen were 0.96 for calcium and 1.61 for phosphorus. In the second place, it was apparently impossible to obtain a homogeneous sample of food for analysis, even when conditions were carefully controlled. Table 4 shows that the composition of duplicate diets collected in the same manner from the same food on the first day of the period varied as much as 6.7% and the average differences ranged from 1.0 to 3.2%. Figure 1 also illustrates the variations between the two analyses on the same period.

In all probability a third factor, the variation in the composition of the same food, also influenced the results because analyzed and calculated values differ. Table 4 shows that the analyzed values in this study varied from the calculated figures as much as 7.3% for calories, 9.4% for protein, 13.9% for calcium and 10.3% for phosphorus but that the average

TABLE 4

Percentage variability in composition of diets

ELEMENT	EXPERIMENT	PROTEIN PER KILOGRAM IN DIET	DIFFERENCE BETWEEN DUPLICATE DIETS		VARIATIONS FROM CALCULATED VALUES		VARIATION OF EACH VALUE FROM MEAN		VARIATION OF PERIOD AVERAGE FROM MEAN		
			Range	Av.	Range	Av.	Range	Av.	Range	Av.	Coefficient of variation
		gm.	%	%	%	%	%	%	%	%	%
Calories	I	3	0.8–4.3	1.8	0.0– 4.9	1.9	0.2–4.1	1.6	0.1–3.3	1.3	1.6
		4	0.1–4.0	1.3	1.2– 7.3	2.7	0.2–5.1	1.9	0.4–3.3	1.9	2.2
	II	3	0.3–3.6	1.7	0.2– 4.5	1.6	0.2–4.1	1.6	0.3–2.9	1.4	1.8
		4	0.2–2.3	1.0	0.2– 3.3	1.6	0.1–3.1	1.6	0.3–2.9	1.6	1.9
Nitrogen	I	3	0.5–4.6	2.0	0.0– 7.8	3.1	0.0–4.9	2.1	0.1–3.3	2.0	2.3
		4	0.3–4.8	1.7	1.2– 4.9	3.0	0.0–5.7	1.5	0.2–3.4	1.5	2.0
	II	3	0.0–4.6	2.0	0.1– 9.4	3.4	0.5–6.3	2.5	0.1–4.1	2.2	2.8
		4	0.7–3.8	2.0	0.1– 4.8	1.8	0.0–3.2	1.3	0.1–2.5	0.9	1.2
Calcium	I	3	0.5–3.9	1.9	0.5– 8.7	4.5	0.0–4.4	1.7	0.3–4.1	1.6	2.0
		4	0.7–2.5	1.4	3.7– 8.4	6.3	0.1–2.8	1.2	0.4–2.3	1.1	1.4
	II	3	0.1–4.3	2.7	4.7–13.9	9.3	0.0–5.2	2.3	0.4–4.5	2.2	2.8
		4	0.3–6.3	3.2	1.0–12.3	7.5	0.0–7.1	2.3	0.5–4.6	2.0	2.5
Phosphorus	I	3	0.3–6.7	2.4	2.4–10.3	6.6	0.1–4.5	1.9	0.3–3.0	1.6	1.9
		4	0.4–3.8	2.3	0.8– 7.5	4.2	0.1–3.5	2.2	0.0–3.3	1.8	2.4
	II	3	1.4–5.3	2.8	0.1– 7.5	3.2	0.2–4.4	2.2	0.8–3.0	1.9	2.2
		4	1.3–3.8	2.3	0.3– 7.3	3.0	0.1–4.7	1.6	0.1–3.0	1.4	1.8

variations were small. It is not surprising that the percentage differences were, in the majority of cases, lower than those which Bray, Hawks and Dye ('34) obtained because their data included many cooked foods the raw weight composition of which was not known. The reason that the differences were lower than those which Bassett and Van Alstine ('35) and Donelson and her associates ('31) obtained, may have been due to the fact that although the average data given by Rose ('32) formed the basis of the calculated values, the

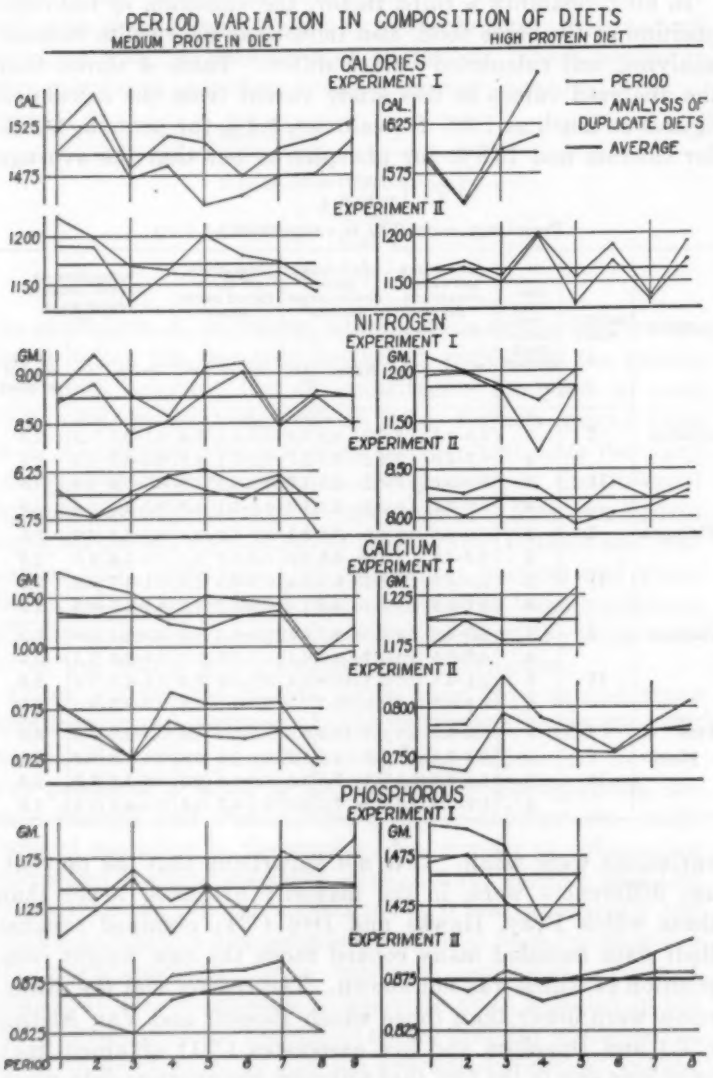
PERIOD VARIATION IN COMPOSITION OF DIETS
MEDIUM PROTEIN DIET
HIGH PROTEIN DIET
CALORIES
EXPERIMENT I
CAL.
1525
1475
1625
1575
PERIOD
ANALYSIS OF DUPLICATE DIETS
AVERAGE
EXPERIMENT II
1200
1150
NITROGEN
EXPERIMENT I
GM.
9.00
8.50
12.00
11.50
EXPERIMENT II
6.25
5.75
8.50
8.00
CALCIUM
EXPERIMENT I
GM.
1.050
1.000
1.225
1.175
EXPERIMENT II
0.775
0.725
0.800
0.750
PHOSPHOROUS
EXPERIMENT I
GM.
1.175
1.125
1.475
1.425
EXPERIMENT II
0.875
0.825
PERIOD
1 2 3 4 5 6 7 8

Figure 1

figures for canned pureéd fruits and vegetables were actual analyses. The variation in the composition of the same food was also illustrated by the fact that there was less difference between the composition of duplicate diets collected from the same food than there was between the highest and lowest value for the eighteen identical diets analyzed (fig. 1). In order to show this difference mathematically, table 4 expresses the variation of all the data from the mean in terms of per cent. The highest of these values, 7.1%, which represents just half of the difference between the highest and the lowest value, was practically the same as the greatest total difference between the composition of the duplicate diets, 6.7%. Therefore, the analysis of any one sample of diet may vary from that of any other sample about twice as much as the difference between the analysis of two samples of the same diet.

Since the values for the duplicate diets usually varied in the same direction from the mean (fig. 1) and showed a period to period variation, the average of the two diets may represent the composition of the food which the children actually received more accurately than either of the separate analyses. Nevertheless, the food which they received may have varied as much as the difference between the duplicate diets or approximately 3.4% from the period average. This period average showed less variation than the values for the individual analyses because the greatest percentage difference from the average of all the data was only 4.6 (table 4). Although the number of cases for statistical treatment were considerably reduced, the coefficients of variation were lowered to values between 1.2 and 2.8.

The variation in the composition of the food is an unavoidable part of every metabolism study, and it seems probable that the metabolic responses of the subjects would reflect these variations in diet. Since the differences would not be the same in successive experiments in one laboratory or in experiments in different laboratories, it is probably necessary to determine the fluctuations in every experiment.

SUMMARY

The procedure used in two balance experiments conducted in this laboratory is described. Some of the precautions taken may suggest improvements in technic which might eliminate some technical errors and make results more constant.

The composition of diets containing the same food and prepared under standard conditions varied not only from period to period but also between duplicate diets collected on the same day. Nevertheless, the coefficients of variation for calories, nitrogen, calcium and phosphorus were not large, ranging between 1.6 and 3.1.

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THE IRON METABOLISM OF NORMAL YOUNG WOMEN DURING CONSECUTIVE MENSTRUAL CYCLES [1]

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THREE FIGURES

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Information concerning the quantitative metabolism of iron in normal women is meager. A search through the literature yields little in regard to the interrelation of blood formation, menstruation, and iron retention. The difficulties of analysis of iron in biological materials, the small amount of iron metabolized by the human organism, and the variation in the physiological state of the subject as a result of the menstrual process have all been reasons for the scarcity of research in this field.

Two studies of the iron metabolism of normal women over short periods of time have been reported in the last 2 years. Ohlson and Daum ('35) studied the iron exchanges of three healthy young women under normal conditions of living and at intervals over 2½ months. Complete collections were made during six periods which ranged from 5 to 15 days in length and included at least one menstrual period for each subject. The diets contained "liberal amounts of protein, calories and protective elements." The average daily intake of iron for all periods was 13.78 mg. and the average daily excretion

[1] Submitted by Ruth M. Leverton in partial fulfillment for the degree of Doctor of Philosophy, The University of Chicago.

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was 14.95 mg. The losses in the menstrual hemorrhage were 25.68 and 41.90 mg. for two subjects and 18.16 and 32.35 mg. for two different periods for the third subject. The authors report that there was no relationship between iron retention and the losses during menstruation. Because their observations were limited to isolated portions of the menstrual cycles of the subjects rather than including complete ones the metabolic picture in regard to iron exchanges is incomplete.

The same is true of the study of Farrar and Goldhamer ('35). One subject, a dietitian, lived on a diet which furnished 9.1 mg. of iron daily for 41 days. The average daily intake for 17 days during the intramenstrual phase was 8.3 mg. and the excretion 8.2 mg. Approximately 33 cc. of blood were lost in menstruation. The balance was not given for the rest of the experimental time.

A complete study of the iron metabolism of women involves certain difficulties. Because 70% of the iron in the body is present in the blood (Sherman, '33) a complete iron balance must consider in so far as possible all blood losses. These may be due to the bleeding of a cut finger, a nosebleed, drawing venous blood for routine analysis, and most particularly to menstruation. Having included these additional paths of possible loss of iron the question arises in calculating a balance of iron as to how these losses should be considered. It may be normal for a woman to be in negative balance during the few days of menstruation and in positive balance at some later time to compensate for this loss or to prepare for the next loss. The results of a brief iron balance study on a woman might depend therefore on the relation of the time of the study to her menstrual period. However, this variable could be obviated by determining her exchanges of iron over a period of time which would include all the changes related to the menstrual process. Thus a menstrual cycle—the recurring period during which the body, particularly the mucous membrane of the uterus, passes through all the changes associated with menstruation—appears to be the most logical time unit for a complete study of the iron balance of women. Also because of the

recognized variations inherent in daily metabolism, figures for the total balance during a cycle would be more significant than calculations for average daily balances.

In view of these facts the present investigation was planned for the purpose of observing by means of a continuous controlled balance experiment the total iron exchanges of normal women during consecutive menstrual cycles. It is hoped that interpretation of the data obtained will contribute to the knowledge of the iron requirement of women, the degree of the iron losses during menstruation, the time and extent of the storage of iron following these periodic losses, and the hemoglobin and red cell content of the blood during different phases of the menstrual cycle.

EXPERIMENTAL PROCEDURES

The investigation constituted a balance study which involved the weighing and analyzing of all the food eaten by the subjects and the collection and analysis of all the excreta during the experiment. Beginning January 2, 1935, the study extended continuously over a period of 110 days for two of the subjects and 140 days for the other two subjects. Four healthy young college women served as subjects both for this study and the study of calcium and phosphorus metabolism which was being conducted at the same time. Three of them were 21 years old and were carrying an ordinary schedule of college work. The fourth one was 27 years old and had a strenuous schedule of laboratory work in connection with this metabolism experiment. Determinations of basal metabolic rate which were made on all subjects 3 consecutive days at the beginning and at the end of the study showed them to be normal in this respect. The menstrual process was reasonably regular in all the subjects and normal according to the subjective criteria of amount and duration of flow.

Daily records were kept of each subject's weight, taken without clothing and before breakfast, of the number of hours of sleep, of any unusual exercise engaged in, and of definite emotional or physical disturbances during the study. Daily

determinations of hemoglobin and red blood cells were made during the greater part of the study and the results are reported in a separate article (Leverton and Roberts, '36). The amount of venous blood which was taken twice during each menstrual cycle in connection with the calcium and phosphorus study was recorded so that the iron lost in this way might be included in the final iron balances.

Planning the dietary for this prolonged metabolic study presented a real problem. One of the purposes of the experiment was to study normal iron metabolism in several subjects when there was constant intake of this mineral from dietary sources over a long period of time. However, directly opposed to the theoretical attainment of this situation was the necessity of adjusting the caloric intake to the energy requirement of each subject, of satisfying individual food likes, and of offering enough variety to make it possible for the subjects to be content to live on this diet with never a break in routine for as long as 5 months. In addition to this was the knowledge of the notorious lack of uniformity in the composition of foods available on the retail market and the problem of securing and keeping the foods from a uniform source of supply. In an attempt to reconcile these conflicting factors without sacrificing too much from the standpoint of having a constant intake nor from the standpoint of having a long-time study the total time of the experiment was divided arbitrarily into consecutive 5-day periods. Then a single foundation dietary was planned and used for every subject during every 5-day period. By varying the order and combination of the different foods during a period a wide variety in the menus could be furnished but the total intake of the foundation dietary was still almost identical in kind and amount for all subjects for all periods. In addition to the main diet certain foods chosen because of their low mineral content were permitted ad libitum. The psychological value of this practice was found to be immeasurable. A list of the kinds and amounts of foods used is given in table 1. During the fourteenth to the twenty-second periods subject A received a daily supplement of 5 mg. of iron in the

form of ferric ammonium citrate, and as part of the calcium and phosphorus study subjects C and D received 353 Steenbock units of vitamin D from biologically standardized cod liver oil daily.

Every attempt was made to secure foods of uniform composition. The canned goods were bought in case lots and except when extremely impractical the perishable foods were bought from the same source throughout the 5 months. All the food was prepared and served in one of the nutrition

TABLE 1

Individual dietary for each 5-day period

FOODS FOUNDATION DIET	AMOUNT IN GRAMS	FOODS FOUNDATION DIET	AMOUNT IN GRAMS	FOODS AD LIBITUM
Orange juice	200	Lettuce	50	White bread
Orance ice	100	Onions	60	Butter
Tomato juice	200	Peas, canned	70	Jelly
Tomato soup concentrate	70	Potatoes, white	200–400	Apples
Grapefruit	100	Potatoes, canned sweet	100	Salad dressing
Peaches, canned	80	Whole wheat bread	300	Jello
Pineapple, canned	80	Doughnuts	60	Plain cookies
Banana	100	Bacon	20	Candy
String beans, canned	70	Beef, lean	400	Coffee
Beets, canned	70	Salmon	90	Tea
Cabbage	100	Eggs, whole	150	Distilled water
Carrots	100	Milk	2400	

laboratories. Since the plan of this study was to approximate an ordinary situation of food preparation and consumption no attempt was made to protect the food from contamination with iron before it was served. Enamel and aluminum ware utensils were used for cooking and although distilled water was used instead of tap water it was only because the subjects drank different amounts and the tap water was known to vary in its mineral content. However, as soon as the food was served to the subjects a portion was placed in a screw top glass jar and then every precaution was taken to keep this sample from collecting additional iron from the air, utensils or containers. This portion was used for making the daily

food composites which represented one-tenth of the amount of all food eaten by each subject. The five daily composites were combined to form a period composite for each subject and this was analyzed for iron.

After the subjects had been on the experimental diet for 10 days, collection of the excreta was begun. All collections of urine and feces were made directly into glass containers. Carmine was given to mark the beginning of each period. All stools for each subject during each 5-day period were combined into a period fecal composite and aliquot portions of daily urine collections were combined into a period urinary composite. The menses were collected on a standard brand of cellulose pad from a single lot which had been sampled at random for blank analysis.

The food and fecal composites and the collection of each menstrual flow were made into smooth brown digests with sulfuric acid by the method of Stearns ('29) with modifications necessary to make it applicable to material which was to be used for iron analyses. Instead of being boiled with the acid the material and the acid were heated at 60 to 70°C. for 2 weeks at the end of which time a homogeneous emulsion resulted. This could be stored indefinitely. The urine was acidified and stored also.

The difficulties involved in the micro-analysis of iron in biological materials are widely recognized and have received much critical attention of late. No one method seems satisfactory from every standpoint nor gives results of unfailing accuracy at the hands of all workers. In this study iron was determined as the thiocyanate by the method developed by Stugart ('31). This method with careful application and certain adaptations gave admirably accurate results. Space does not permit reporting here the results of the extensive laboratory work and the meticulous routine necessary to establish definitely the adequacy of this method. Every effort was made to prevent the contamination of the samples by iron-laden dust particles. A small protected laboratory of wood and glass was constructed within the larger chemistry laboratory in order to have a place where the samples could be

handled without their accumulating extraneous iron. The method of treatment of the samples consisted in drying and carefully ashing at about 400°C. aliquot portions of the brown digests and of the urine composites, and dissolving the ash in hydrochloric acid. Aliquot portions of the ash solution were then hydrolyzed with acid to change the pyrophosphates to orthophosphates. The iron present was converted to ferric thiocyanate and its amount determined by colorimetric comparison in amyl alcohol solution with a standard solution of iron. At first triplicate determinations were made on each of the ash solutions of three aliquots from each digest but later this number was reduced to duplicate determinations on the ash solution of each of two aliquots from each digest.

For several reasons only total iron was determined in food and excreta in this study in spite of the fact that recent research indicates that iron must exist in the inorganic form to be available for hemoglobin regeneration. Among these was the fact that the most satisfactory method for determining inorganic iron requires the use of individual samples of fresh food material and not composites in which the lack of a homogeneous mixture makes accurate sampling impossible. Daily analysis of individual samples would have been prohibitive in number because of the variety of foods used in each period and the different combinations in which they were prepared. Since the method of preparation can have such a variable effect on the inorganic iron in foods, figures for the average available iron content secured from analysis of a few samples would have been little less than approximations. Moreover, the non-availability of iron in the organic form for hemoglobin building may be due to its incomplete absorption from the intestine (Vahlteich et al., '36). The problem of absorption and re-excretion of iron by the gastro-intestinal tract is not within the realm of a balance experiment and therefore the determination of inorganic or available iron in the feces would be of no more value than one of total iron. Finally, availability has as yet only been demonstrated to apply to the utilization of iron for hemoglobin formation following very

severe anemia and since iron is an essential constituent of animal protoplasm, even though 70% of it exists as hemoglobin, it seemed more pertinent in this study of iron exchanges in normal women to determine total rather than available iron.

RESULTS

The data from this study of iron metabolism will be presented in the following order: 1) the balance between the average daily intake and fecal and urinary excretion of iron for each 5-day period, for each menstrual cycle, and for the entire study; 2) the iron losses in the menses; 3) the balance for each menstrual cycle including the iron lost in the menses; and 4) the actual balance for each cycle and for the entire study when the iron lost through the drawing of venous blood samples and through a nosebleed for one subject is considered.

Balance between food and excreta. The average daily intake, excretion and balance of iron of the four subjects during the consecutive 5-day periods of the metabolism study were calculated from the results of the analysis of the composites of the food, urine and the feces for each period. The results are presented in table 2 expressed in total milligrams of iron per day and in milligrams of iron per kilogram of body weight per day for each subject for each 5-day period. In this table the excretion includes both urinary and fecal iron. Because the daily quantity of iron in the urine was small (0.19, 0.17, 0.29 and 0.19 mg. for subjects A, B, C and D, respectively) and constant it seemed justifiable to add it to the many times larger fecal excretion and thus report only one figure for the excreta. Even though the daily urinary excretion of iron was not great enough to be significant in determining a daily balance such a loss could not be disregarded over a period of several months.

When the average daily amount of iron ingested is compared with the average daily amount excreted in the feces and urine for the entire metabolic period subjects A, B and C were in positive balance and subject D in slight negative balance.

TABLE 2

Average daily intake, excretion and balance of iron of four subjects during consecutive 5-day periods of the metabolism study

SUBJECT	PERIOD	AVERAGE MILLIGRAMS IRON PER DAY			AVERAGE MILLIGRAMS IRON PER KILOGRAM PER DAY		
		Intake	Excretion	Balance	Intake	Excretion	Balance
A	A	9.86	8.11	+1.75	0.166	0.137	+0.029
	B	12.08	12.83	—0.75	0.204	0.216	—0.012
	C	10.56	10.35	+0.21	0.180	0.177	+0.003
	D	10.16	13.15	—2.99	0.176	0.224	—0.048
	E	12.33	8.11	+4.22	0.210	0.138	+0.072
	F	11.36	9.79	+1.57	0.194	0.167	+0.027
	G	11.84	11.95	—0.11	0.200	0.203	—0.003
	H	14.16	12.67	+1.49	0.241	0.216	+0.025
	I	12.48	12.03	+0.45	0.212	0.205	+0.007
	J	14.00	11.47	+2.53	0.238	0.195	+0.043
	K	11.12	11.07	+0.05	0.227	0.187	+0.040
	L	13.60	12.67	+0.93	0.232	0.216	+0.016
	M	13.44	9.63	+3.81	0.230	0.164	+0.066
	N	17.68	19.39	—1.71	0.300	0.331	—0.031
	O	18.16	25.79	—7.63	0.308	0.437	—0.129
	P	18.16	16.83	+1.33	0.308	0.284	+0.024
	Q	17.04	21.59	—4.55	0.288	0.365	—0.077
	R	16.16	8.29	+7.87	0.272	0.140	+0.132
	S	16.56	14.35	+2.21	0.279	0.242	+0.037
	T	17.28	16.52	+0.76	0.290	0.277	+0.013
	U	17.52	16.92	+0.60	0.295	0.285	+0.010
	V	12.54	12.67	—0.13	0.210	0.212	—0.002
	W	11.60	12.63	—1.03	0.193	0.210	—0.017
	X	10.24	7.87	+2.37	0.170	0.132	+0.038
	Y	11.52	11.71	—0.19	0.190	0.198	—0.008
	Z	12.16	7.15	+5.01	0.204	0.120	+0.084
	Average	13.61	12.89	+0.72	0.231	0.220	+0.011
B	A	10.85	2.30	+8.55	0.180	0.037	+0.043
	B	10.64	10.39	+0.25	0.174	0.172	+0.002
	C	10.96	13.77	—2.81	0.178	0.223	—0.045
	D	10.00	12.41	—2.41	0.164	0.203	—0.039
	E	9.84	8.25	+1.59	0.163	0.136	+0.027
	F	12.56	8.81	+3.75	0.209	0.147	+0.062
	G	10.64	9.85	+0.79	0.175	0.163	+0.012
	H	14.56	11.13	+3.43	0.240	0.183	+0.057
	I	12.56	10.01	+2.55	0.208	0.165	+0.043
	J	12.44	12.89	+0.45	0.205	0.211	—0.006
	K	11.20	14.49	—3.29	0.185	0.240	—0.055
	L	12.88	9.53	+3.35	0.213	0.158	+0.055
	M	12.96	9.61	+3.35	0.214	0.159	+0.055
	N	12.00	14.01	—2.01	0.200	0.234	—0.034
	O	11.92	7.37	+4.55	0.198	0.122	+0.076
	P	13.84	10.65	+3.19	0.227	0.175	+0.052
	Q	13.04	11.45	+1.59	0.216	0.189	+0.027
	R	11.12	10.49	+0.63	0.183	0.173	+0.010
	Average	11.87	10.39	+1.48	0.196	0.172	+0.024

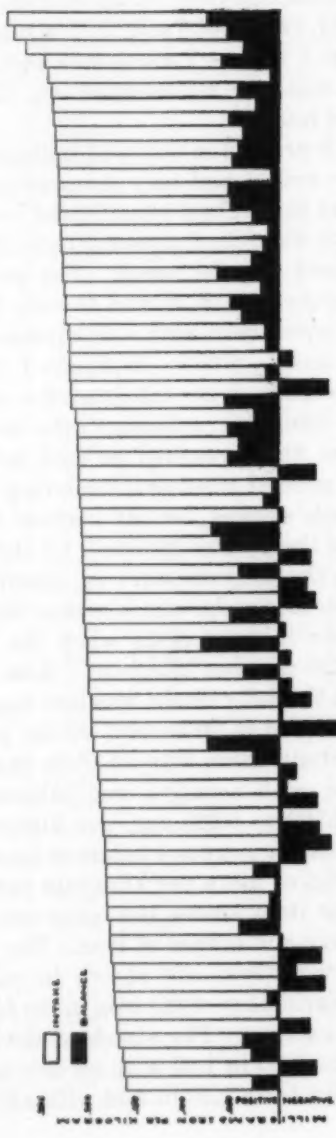
TABLE 2—*Continued*

SUBJECT	PERIOD	AVERAGE MILLIGRAMS IRON PER DAY			AVERAGE MILLIGRAMS IRON PER KILOGRAM PER DAY		
		Intake	Excretion	Balance	Intake	Excretion	Balance
C	A	11.12	9.01	+2.11	0.242	0.198	+0.044
	B	10.08	9.49	+0.59	0.222	0.208	+0.014
	C	10.72	8.37	+2.35	0.235	0.185	+0.050
	D	10.48	9.17	+1.31	0.230	0.201	+0.029
	E	10.72	8.35	+2.37	0.234	0.183	+0.051
	F	13.04	10.61	+2.43	0.287	0.232	+0.055
	G	9.44	11.17	—1.73	0.206	0.244	—0.038
	H	11.76	8.93	+2.83	0.255	0.194	+0.061
	I	10.48	9.57	+0.91	0.228	0.208	+0.020
	J	10.03	7.53	+2.50	0.221	0.163	+0.058
	K	9.44	7.57	+1.87	0.202	0.165	+0.037
	L	8.72	10.61	—1.89	0.190	0.231	—0.041
	M	10.16	9.49	+0.67	0.222	0.207	+0.015
	N	10.72	10.21	+0.51	0.232	0.221	+0.011
	O	11.60	11.25	+0.35	0.251	0.243	+0.008
	P	9.60	11.41	—1.81	0.212	0.251	—0.039
	Q	10.64	8.17	+2.37	0.234	0.180	+0.054
	R	8.24	7.73	+1.51	0.182	0.171	+0.011
	S	10.48	9.25	+1.23	0.233	0.205	+0.028
	T	8.48	8.53	—0.05	0.187	0.189	—0.002
	U	10.80	9.01	+1.79	0.238	0.199	+0.039
	V	10.48	10.13	+0.35	0.234	0.226	+0.008
	W	9.84	12.13	—2.29	0.221	0.273	—0.052
	X	7.92	8.69	—0.77	0.176	0.193	—0.017
	Y	8.00	6.85	+1.15	0.184	0.157	+0.027
	Z	7.76	7.89	—0.13	0.183	0.187	—0.004
	Average	10.03	9.32	+0.71	0.221	0.204	+0.017
D	D	10.16	12.43	—2.27	0.186	0.228	—0.042
	E	10.80	14.99	—4.19	0.200	0.277	—0.077
	F	10.88	11.47	—0.59	0.202	0.214	—0.012
	G	9.36	11.15	—1.79	0.173	0.206	—0.033
	H	12.08	11.31	+0.77	0.222	0.208	+0.014
	I	11.28	4.71	+6.57	0.207	0.086	+0.121
	J	11.44	21.15	—9.71	0.209	0.387	—0.178
	K	16.16	11.95	+5.21	0.295	0.219	+0.076
	L	15.04	13.09	+1.95	0.275	0.239	+0.036
	M	12.16	12.89	—0.73	0.222	0.236	—0.014
	N	11.52	13.29	—1.77	0.209	0.241	—0.032
	O	11.76	9.43	+2.33	0.214	0.171	+0.043
	P	11.60	10.69	+0.91	0.211	0.194	+0.017
	Q	11.28	8.39	+2.89	0.206	0.153	+0.053
	R	10.16	11.71	—1.55	0.185	0.213	—0.028
	Average	11.71	11.91	—0.20	0.214	0.218	—0.004

As given in table 2 the average daily intake of iron for subjects A, B, C and D was 13.61, 11.87, 10.03 and 11.71 mg. and the average daily balance was + 0.72, + 1.48, + 0.71 and — 0.20 mg., respectively. These balances represented 5.3, 12.5, 7.1 and 1.7% of the respective intakes.

When the data are expressed in terms of milligrams of iron per kilogram of body weight and only the averages are considered it appears that the highest retention of iron occurred on the lowest intake but when the figures for individual periods are analyzed other facts may be noted. The average daily intakes of iron for subjects A, B, C and D were 0.231, 0.196, 0.221 and 0.214 mg., respectively, with corresponding balances of 0.011, 0.024, 0.017 and — 0.004. In figure 1 the average daily intake of iron from food per kilogram for each subject during each period is plotted in relation to the average daily retention per kilogram which resulted on each intake. Here it may be seen that in general whether considering all subjects for all periods or each subject for all periods the greater retentions occurred on the greater intakes. Of the twenty-six periods during which the subjects were in negative balance, thirteen occurred when the intake was less than 0.200 mg. per kilogram and the other thirteen cases when the intake was between 0.200 and 0.225 mg. per kilogram. Also during the fifty-two periods when the daily intake was less than 0.225 mg. a negative balance occurred in 26 or 50% of the periods. In other words, in this study there was an even chance of the body being in positive or in negative iron balance when the intake ranged from 0.160 to 0.225 mg. per kilogram, but it is significant to note that no negative balances occurred when the intake was 0.225 mg. or more per kilogram per day.

Observation of these data shows the occurrence of many variations in both intake and output of iron. The extent and significance of these variations are shown in table 3. As might be expected the variability of the iron in the food is much less than that in the excreta. The standard deviations for iron intakes range from 1.20 to 1.65 with an average of 1.35; those for iron excretions from 1.30 to 4.34 with an average of



RELATION OF AVERAGE DAILY INTAKE TO AVERAGE DAILY RETENTION OF IRON, FOR ALL SUBJECTS AS CALCULATED PER KILO OF BODY WEIGHT.

Figure 1

2.96. The average coefficients of variation are 11.9 and 25.8 for iron of food and excreta respectively. For three subjects the variability of the output is at least twice that of the intake and for the fourth subject the two are practically the same.

The variabiliy of the daily balances is best demonstrated by figure 2 which shows graphically the average daily balance of each subject for each 5-day period as given in table 2, for each 10-day period, and for each menstrual cycle. There are periods of negative and positive iron balance for each of the

TABLE 3

Statistical constants

SUBJECT	MEAN	RANGE		STANDARD DEVIATION	COEFFICIENT OF VARIATION
		Lowest	Highest		
Average daily food intake					
	mg.	*mg.*	*mg.*	*mg.*	
Subject A	11.93[1]	9.86	14.16	1.20	10.1
Subject B	11.87	9.84	14.56	1.24	10.5
Subject C	10.03	7.76	13.04	1.30	13.0
Subject D	11.71	9.36	16.16	1.65	14.1
Average daily total excretion					
Subject A	12.89	7.15	25.79	4.34	33.6
Subject B	10.39	2.30	14.49	2.94	28.3
Subject C	9.32	6.85	12.13	1.30	13.9
Subject D	11.90	4.71	21.15	3.27	27.4

[1] Does not include iron from ferric ammonium citrate.

subjects during the metabolism study but no consistent regularity in the order of their occurrence can be detected. Of particular note is the independence of retention with respect to the menstrual periods. The results indicate no regularity in the way the body compensated for the menstrual losses of the subjects.

Effect of ferric ammonium citrate supplement. Beginning with the fourteenth consecutive 5-day period and continuing through the twenty-second period subject A was given a daily supplement of 5 mg. of iron in the form of ferric ammonium

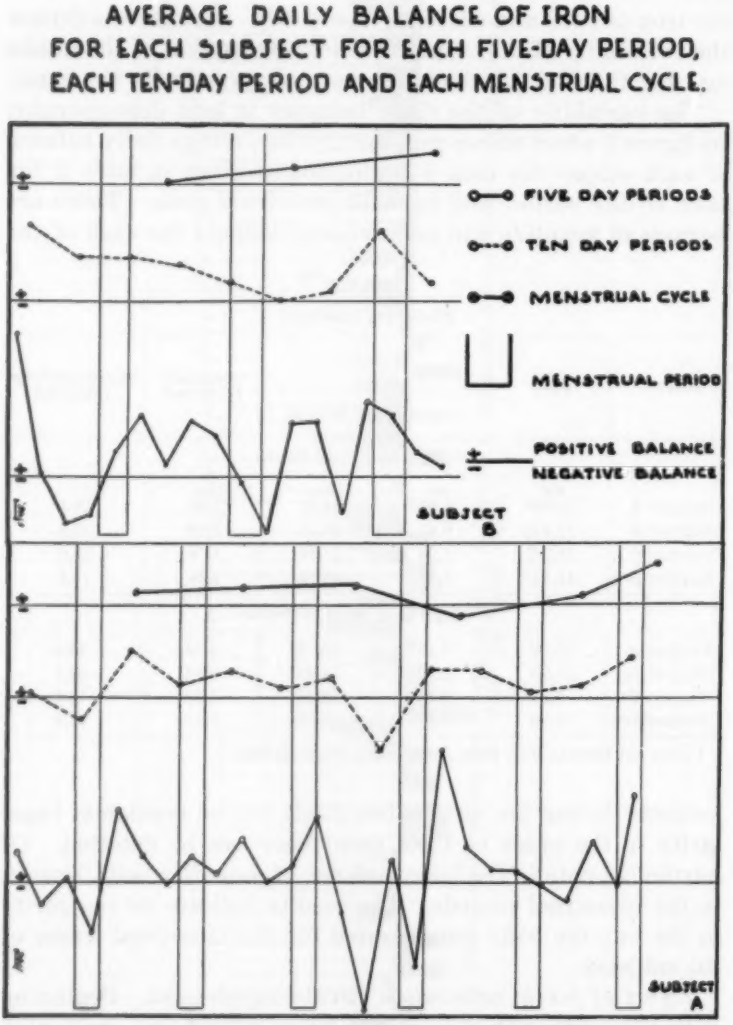

AVERAGE DAILY BALANCE OF IRON
FOR EACH SUBJECT FOR EACH FIVE-DAY PERIOD,
EACH TEN-DAY PERIOD AND EACH MENSTRUAL CYCLE.
FIVE DAY PERIODS
TEN DAY PERIODS
MENSTRUAL CYCLE
MENSTRUAL PERIOD
POSITIVE BALANCE
NEGATIVE BALANCE
SUBJECT B
SUBJECT A

Figure 2

citrate. The effect of this is shown in table 4 where the average daily intake, excretion and balance of iron for the periods before and after the iron therapy are compared with those

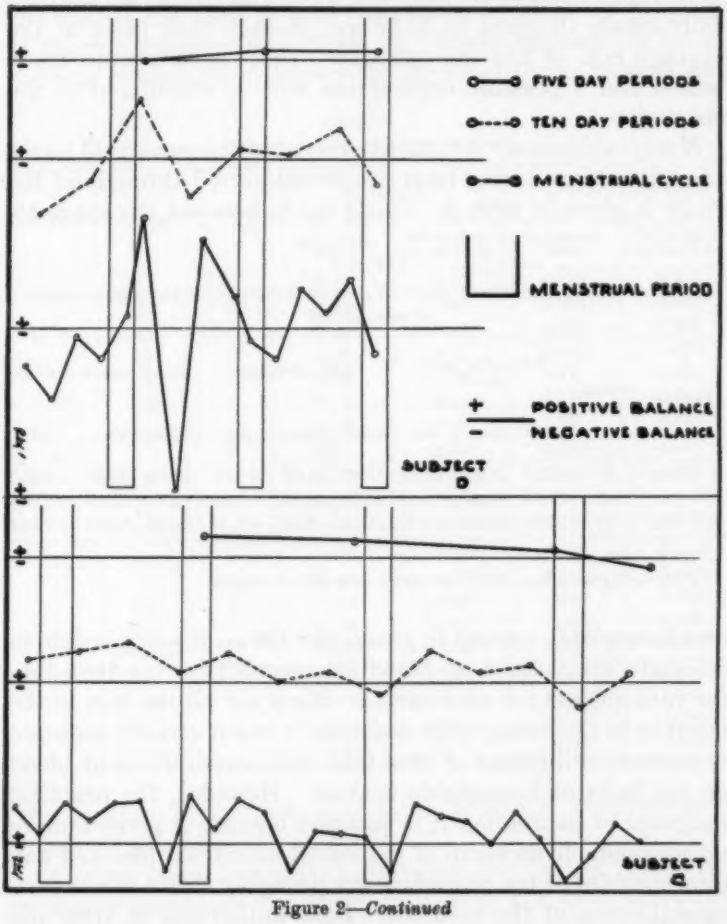


Figure 2—*Continued*

for the periods of iron therapy. During the first thirteen periods of the study subject A had an average positive balance between food and feces and urine of + 1.01 mg. of iron per

day on an average intake of 12.07, but when the average intake was increased to 16.79 by the addition of a solution of ferric ammonium citrate this balance was reduced to — 0.10 mg. per day. When this iron therapy was discontinued and the average daily intake dropped to 11.38 mg. storage took place at the average rate of 1.54 mg. per day. These results were unexpected and a possible explanation will be considered in the discussion.

Menstrual losses. A complete record of the menstrual losses of each subject during each menstrual period throughout the study is given in table 5. Using the figures for the subject's

TABLE 4

Effect of a daily supplement of ferric ammonium citrate on the iron exchange of one subject

PERIODS	TOTAL DAYS	TOTAL PER 5-DAY PERIOD			DAILY AVERAGE			PER KILOGRAM PER DAY		
		Intake	Excr.	Bal.	Intake	Excr.	Bal.	Intake	Excr.	Bal.
A-M inc.[1]	65	60.37	55.32	+5.05	12.07	11.06	+1.01	0.208	0.188	+0.020
N-V inc.[2]	45	83.95	84.47	—0.52	16.79	16.89	—0.10	0.283	0.290	—0.007
W-Z inc.[1]	20	56.90	49.20	+7.70	11.38	9.84	+1.54	0.189	0.165	+0.024

[1] No supplement.

[2] Five milligrams iron daily as ferric ammonium citrate.

own hemoglobin content in grams per 100 cc. it was possible to calculate the volume of blood represented by the iron loss for each subject for each period. Since not all the iron in the blood is in the hemoglobin molecule it is not strictly accurate to convert milligrams of iron into cubic centimeters of blood on the basis of hemoglobin content. However, for practical purposes of expression it is justified because it gives a more understandable measure of the actual menstrual loss and any discrepancies of the procedure are probably within the experimental error of the methods. Both milligrams of iron and cubic centimeters of blood are also calculated on the basis of body weight. Subjects A, B, C and D lost an average of 14.26, 22.84, 11.13 and 13.80 mg. of iron, or 30.01, 50.78, 26.48

and 29.68 cc. of blood, respectively, during each menstrual period. The most striking fact shown by the individual figures is the constant amount of iron lost in the different menstrual periods of the same subject. The variation from one period to the next is less than 10% in the periods of subjects B, C and D though greater for subject A. There appears to be no definite relationship between the iron lost in the menses and

TABLE 5

Menstrual losses of four subjects

SUBJECT	PERIOD	DURATION IN DAYS	TOTAL LOSS		LOSS PER KILOGRAM	
			Milligrams iron	Cubic centimeters blood	Milligrams iron	Cubic centimeters blood
A	I	5	10.46	23.75	0.178	0.403
	II	5	16.80	35.91	0.286	0.611
	III	6	16.40	35.05	0.279	0.597
	IV	5	11.86	23.57	0.201	0.399
	V	6	12.96	25.89	0.217	0.434
	VI	5	17.10	35.92	0.288	0.605
	Average		14.26	30.01	0.241	0.510
B	I	7	22.01	51.62	0.361	0.848
	II	7	22.37	49.73	0.369	0.821
	III	6	24.13	50.98	0.399	0.843
	Average		22.84	50.78	0.380	0.837
C	I	8	10.86	27.49	0.238	0.603
	II	7	11.26	26.98	0.245	0.587
	III	6	10.86	25.29	0.240	0.558
	IV	6	11.56	26.15	0.264	0.597
	Average		11.13	26.48	0.247	0.586
D	I	5	13.26	29.86	0.245	0.551
	II	5	14.52	31.17	0.266	0.570
	III	4	13.62	28.01	0.248	0.510
	Average		13.80	29.68	0.253	0.544

the duration of the period, or to the length of the cycle or to the body weight of the subject.

Another blood loss must be recorded for subject A who had a severe nosebleed the night preceding her fifth menstrual period. The blood was collected on cellulose pads and treated in the same way as the menses. The iron content of the blood loss was 36.57 mg. which was equivalent to about 73 cc. of

her blood. She also had had slight nosebleeds during menstrual periods II and III but because the loss was small the blood was added to the menses for those periods. After the severe loss preceding period V her nostril was cauterized and there was no recurrence of this bleeding.

TABLE 6

Balance of iron for each menstrual cycle for each subject

CYCLE	NUMBER OF DAYS	AVERAGE DAILY			BALANCE FOR CYCLE	LOSS IN MENSTRUATION	FINAL BALANCE	LOSS IN VENOUS BLOOD	ACTUAL BALANCE
		Intake	Excretion	Balance					
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Subject A									
I	22	11.22	10.91	+0.31	+6.72	10.46	—3.74	9.69	—13.43
II	21	12.66	11.68	+0.98	+20.49	16.80	+3.69	10.98	—7.29
III	23	13.96	12.97	+0.99	+22.99	16.40	+6.59	4.68	+1.91
IV	21	17.34	17.95	—0.61	—12.69	11.86	—24.55	11.07	—35.62
V	24	15.04	14.57	+0.47	+11.44	12.96	—1.52	36.57[1] 5.01	—43.10
VI	15	11.31	8.91	+2.40	+36.00	17.10	+18.90	4.76	+14.14
	Total				+84.95	—85.58	—0.63	—82.76	—83.39
Subject B									
I	29	10.79	10.64	+0.15	+4.24	22.01	—17.77	9.38	—27.15
II	27	12.57	11.48	+1.09	+29.53	22.37	+7.16	12.15	—4.99
III	28	12.52	10.55	+1.97	+55.24	24.13	+31.11	0.0	+31.11
	Total				+89.01	—68.51	+20.50	21.53	—1.03
Subject C									
I	30	10.80	9.50	+1.30	+39.06	10.86	+28.20	5.93	+22.27
II	35	10.18	9.20	+0.98	+34.49	11.26	+23.23	12.52	+10.71
III	39	10.00	9.52	+0.48	+18.85	10.86	+7.99	9.59	—1.60
IV	20	8.38	8.89	—0.51	—10.20	11.56	—21.76	6.63	—28.39
	Total				+82.20	—44.54	+37.66	34.67	+2.99
Subject D									
I	27	10.72	10.84	—0.12	—3.25	13.26	—16.51	4.44	—20.95
II	26	13.17	12.70	+0.47	+12.22	14.52	—2.30	9.32	—11.62
III	25	11.26	10.70	+0.56	+14.05	13.62	+0.43	9.72	—9.29
	Total				+23.02	—41.40	—18.38	23.48	—41.86

[1] Loss in nosebleed.

Balances for complete menstrual cycles. In order to give the most complete picture of the iron exchanges in the subjects the data are presented for each menstrual cycle, the logical unit of time for which to calculate an iron balance. In table 6

the average daily intake, excretion and balance of iron is calculated for each cycle and then the total balance for each cycle is presented together with loss in the menses and the balance between these is shown. This is designated as the 'final' balance for each subject, since it is the figure which would be of chief consideration in the usual iron metabolism of women. Since the loss in the venous blood samples and the loss by subject A in a severe nosebleed cannot be disregarded they have been subtracted from the final balance figures and the result designated as the 'actual' balance for the subjects of this study.

It may be noted from the figures given in table 6 that during thirteen of the sixteen menstrual cycles studied the average daily intake exceeded the average daily excretion. The average daily positive balances range from 0.31 mg. to 2.40 mg. with a mean value of 0.93 mg. for all subjects. During the other three cycles there were daily negative iron balances of 0.61, 0.51 and 0.12 mg. between intake and excreta.

The subjects did not always retain enough iron from their intakes to compensate for that lost in the menses. During only nine of the sixteen cycles studied was the total positive balance between intake and excretion sufficient to cover completely the iron lost in the menstrual flow and thus place the subjects in final iron equilibrium or in positive balance. In three other cycles the final negative balances were probably small enough to be negligible, 3.74, 1.52 and 2.30 mg. The final balances for the remaining four cycles were — 16.51, — 17.77, — 21.76 and — 24.55 mg.

Calculation of the actual iron balance of these subjects includes the loss of iron due to the taking of blood samples as well as that in the feces, urine and menses. In only six of the sixteen menstrual cycles studied were the subjects able to compensate for the additional iron lost from the body in the venous blood samples. When the entire time of the study is considered only subjects B and C were near actual iron equilibrium. Subject B accomplished this at one fell swoop in the last cycle by retaining 31.11 mg. which practically compensated for the 32.14 mg. lost in the two previous cycles.

Conversely subject C stored 32.98 mg. in cycles I and II and lost 29.99 mg. in cycle III and the first 20 days in cycle IV. Subject A retained enough to balance the loss in the venous blood samples only during cycles III and VI and subject D never did this. Subject A was in actual negative iron balance to the extent of 83.39 mg. and subject D of 41.86 mg. for the entire study.

Total metabolic picture for the entire study. Since much of the value of this study of iron metabolism lies in the fact that it was continuous over several months a presentation of the complete metabolic picture is important. In figure 3 the total number of milligrams of iron in the food are compared with the total loss of iron in the feces, urine and venous blood samples for each subject for the entire time of the experiment. Under ordinary conditions the final balances between the iron intake and the sum of the iron losses in the feces, urine and menses would give the complete picture of the measurable iron metabolism. Considering just these pathways of loss subjects B and C were in slight positive balance, subject A was in equilibrium, and subject D in negative balance during the entire study. However, the subjects in this study lost additional iron due to the taking of venous blood samples for calcium and phosphorus analysis. Thus when this is considered the 'actual' balances show that subjects B and C were in equilibrium and subjects A and D were in negative balance.

The intakes and losses are calculated also on the basis of milligrams of iron per kilogram per day and shown also in figure 3. By thus ruling out differences due to the varied length of time on the balance study and to unequal body weights the results for all the subjects are comparable. The sum of the losses in the feces, urine and menses for subjects A (omitting the periods of iron therapy), B, C and D were 0.201, 0.192, 0.213 and 0.219 mg. of iron per kilogram per day, respectively. The average is 0.206 mg. These figures will be used later in the calculations of minimum requirement of iron for these subjects and for other normal women.

Hemoglobin and red cell content of the blood. The results of the daily determinations of hemoglobin and red cell content have been reported in a separate article (Leverton and Roberts, '36). Analysis of the data shows the occurrence of daily variations in both hemoglobin and red cells, the majority of which are not greater than the experimental error. The standard deviation for the entire series is 0.90 for hemoglobin and 0.31 for red cells. There was a definite upward trend in the hemoglobin value of every subject during the entire period.

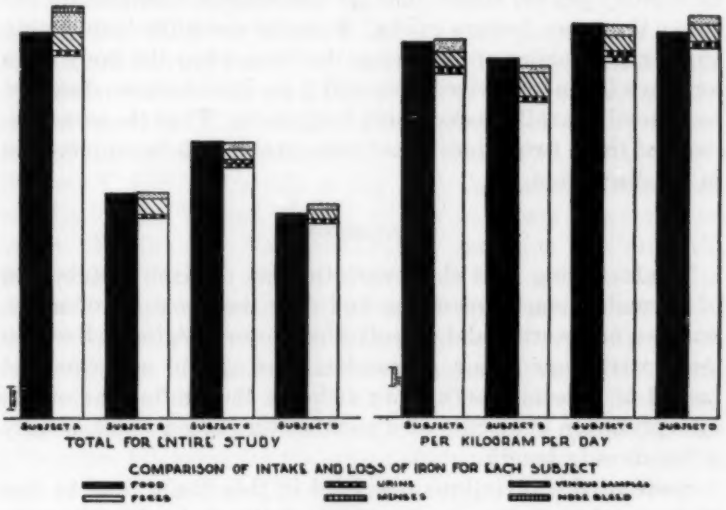


Figure 3

The values increased from 12.95, 12.54, 11.62 and 13.06 gm. per 100 cc. for subjects A, B, C and D, respectively, for the first menstrual cycle to 14.00, 13.92, 13.00 and 14.30 for the last cycle in the study. The red cell count remained remarkably constant for all subjects throughout the experiment. In subject A when 5 mg. of iron from ferric ammonium citrate was given daily during two menstrual cycles the red cell content increased 0.5 million per cubic millimeter and the hemoglobin content increased 1 gm. per 100 cc. These higher

values were not maintained when the citrate was discontinued. From the data presented it appears that there is no measurable or consistent effect of the process of menstruation upon the daily values of either hemoglobin or red cells in the subjects studied. Although occasional marked daily variations in the blood values occur they do so irrespective of the different phases of the menstrual cycle.

A comparison of the average daily iron balance of each subject with the average hemoglobin content of her blood for each 5-day period shows that no measurable relationship between these two factors exists. In many cases the hemoglobin value rises during or following the time when the body is in negative balance or vice versa and a positive balance does not necessarily parallel hemoglobin formation. That the measurement of these two values is not comparable will be emphasized in the discussion.

DISCUSSION

The foregoing data show variations in the iron metabolism of normal young women who had only the demands of maintenance, and were under a controlled dietary regime. Because these variations occur at random during the experimental period of several months they indicate the limitations of the interpretation of results of a metabolism period which is only a few days in length.

Some of the variations observed in this study may be due to fluctuations in the iron content of the diet. Despite precautions taken to control this factor, differences in the iron content of foods due to manufacture, preservation and cooking made some variation inevitable. Also in the present study it was advisable to permit some foods ad libitum and although chosen carefully they were not iron-free and so caused some of the variation observed in the iron intakes of the subjects during the different periods. Marked fluctuation in the iron content of supposedly constant diets, and of samples of the same food taken at different times from different sources, have been reported also by Coons ('35), Toscani and Reznikoff

('34), and Davidson and LeClerc ('36). Such findings together with those of the present study indicate that analysis of each diet as eaten by each subject is the only accurate procedure for balance experiments.

Even though variations occur in the iron intakes they are not as great as the irregularities in the iron excretions and thus in the calculated balances. To what extent the rate of intestinal motility influenced the excretion of iron is speculative. All the subjects had reasonably regular habits of elimination but they did not have the same rhythm or rate of defecation. Subjects A and B had an average of three bowel movements during each 5-day period, and the carmine did not appear in their stools until the second or third morning after it was ingested. The coefficient of variation of the iron content of the excreta in these cases was 38 and 28, respectively. Subject C had two stools a day and the carmine appeared usually within 12 and always within 24 hours after it was taken. In this case the coefficient of variation was only 13, the same as for her intake. Subject D had one movement a day and the carmine always appeared the first morning after it was taken, but even with this regularity the coefficient of variation was 27. It is of interest that subjects A (omitting the periods of iron therapy) and B with a slower intestinal motility had an average daily excretion of 0.177 and 0.172 mg. of iron per kilogram for the entire study and subjects C and D with a more rapid motility had an average daily excretion of 0.204 and 0.214 per kilogram, 18% higher than for A and B. No relationship was found to exist between variations in the fiber content of the diet—entailed by the fact that apples were permitted ad libitum—and variations in the iron excretion.

Evidence of the influence of variations on the interpretation of the results of a metabolism study is demonstrated best by figure 2. In this average total daily retentions are charted for periods of different lengths—5 days, 10 days, and complete menstrual cycles. Careful study of the chart shows that the results from an isolated 5-day period or even a 10-day period would give a very different picture of the iron metabolism of these women than do the results of a continuous study

over a longer period of time. These variations in iron balances when the subjects were under reasonably controlled conditions for several months together with the fact that the variations did not lessen as the study proceeded offer striking evidence of the need for long and continuous metabolism periods if a true record of the body's activity is to be obtained.

It is of interest to compare the average menstrual losses of iron for the subjects in this study with those reported in the literature. Barer and Fowler ('36) have studied the losses of 100 normal individuals during menstruation and found an average iron loss of 19.54 mg. per period. When translated into blood losses on the basis of individual hemoglobin content the average was 50.55 cc. and the standard deviation 25.73 cc. Fifty per cent of her subjects lost between 23.21 and 68.43 cc. of blood in a period. The losses in the present study were 11, 13, 14 and 22 mg. of iron, which represented blood losses ranging from 24 to 52 cc. with an average of 34 cc. Barer's figures and those from this study show that when the amount of blood lost in the menstrual flow is calculated from actual analysis the figures are markedly lower than the usual estimates of 150 cc. to 600 cc. given in physiology textbooks.[2] This increasing knowledge that the normal menstrual losses are probably much less than were heretofore thought and therefore less alarming from the standpoint of the total quantity of blood lost is indeed acceptable. The very constant losses during different periods for the same subject in this study might suggest that in normal women on an adequate regular diet the menstrual loss is definite and characteristic for each individual. This, however, would not be concluded from the findings of Barer who reports marked variation from period to period and more variations for the subjects whose menstrual losses were greater than the average.

The results of increasing the iron intake of subject A by the addition of ferric ammonium citrate were unexpected since reports in the literature indicate that this form of iron is

[2] It has been suggested that gynecologists are prone to disregard these figures and accept the Hoppe-Seyler figure of 37 cc. as nearer the true value.

readily available to the body. The average daily negative balance of 0.10 mg. between food and feces and urine during the periods of increased iron intake is hardly large enough to be significant, but the change in daily iron retention from + 1.01 to — 0.10 when it was added and then from — 0.10 to + 1.54 when it was withdrawn is striking. Although the astringent effect on the intestinal tract of some iron salts is known it has not been reported in the case of ferric ammonium citrate and was not expected of the 3 cc. of 0.95% solution given daily in this case.

The concurrent hemoglobin values for subject A during this time are not in accord with her condition of negative iron balance or even with a condition of equilibrium. During the cycles when she was receiving additional iron her hemoglobin increased from 13.8 to 14.8 gm. per 100 cc. of blood and then dropped to 14.0 when the supplement was withdrawn. The statistical significance of this increase has been considered in the original report (Leverton and Roberts, '36). Hemoglobin formation requires iron, but at no time during the entire study had subject A retained enough from either food or food plus supplement to account for the iron storage represented by the increase in the total hemoglobin content of her blood. This means that the hemoglobin was formed at the expense of the bodily stores of iron and that these stores were not replenished during the experimental regime. Because of the recognized role of copper in hemoglobin formation, it was suspected that the contamination of the ferric ammonium citrate by copper might be one cause for the rise in hemoglobin value. This possibility was precluded, however, when the copper content of the 5-day foundation dietary was calculated and found to be as high as 6 mg. Schlutz, Morse and Oldham ('33) and Fowler and Barer ('35) report large iron retentions and the latter coincident hemoglobin regeneration following the administration of ferric ammonium citrate. The differences between their results and the results of this supplementary iron feeding experiment may be due to several factors. Fowler and Barer used as their subjects six patients with

hypochromic anemia. Although they do not state the amount of the supplement given they report a storage as high as 6.27 gm. of iron in 24 days which would indicate a daily dose of over 250 mg. Likewise Schlutz, Morse and Oldham fed an infant a daily dose of 100 mg. of iron in this form plus 0.75 mg. of copper and found a large iron retention though practically no hemoglobin regeneration, but here again the subject was markedly anemic. Certainly these are not conditions comparable to those of subject A who was receiving only a 5 mg. supplement of iron and who was, moreover, normal when the supplement was begun.

A possible explanation of the failure of subject A to store the additional iron given her orally is found in the result of the study by Heath et al. ('32) of hemoglobin regeneration following oral and parenteral administration of ferric ammonium citrate to patients with hypochromic anemia. He found that it required a daily dose of 1000 mg. of metallic iron when given orally as ferric ammonium citrate to produce the same blood-building effect as a daily dose of 32 mg. of metallic iron when given parenterally in the same form. That absorption of the iron in this form was the limiting factor appears likely though this is not in accord with the results of the studies on the availability of iron from ferric ammonium citrate.

The lack of correlation between the iron balances and the hemoglobin values for the different periods is not unexpected when the difference in the amount of iron required to make a significant change in these values is calculated. For subject A a change of 140 mg. in the amount of iron circulating in the blood stream would be required to vary the hemoglobin value 1 gm. per 100 cc., and then because of the method used for its determination such a change would be considered significant only if it resulted from many analyses over a period of several weeks. This change nevertheless involves ten times the amount of iron lost during a single menstrual period by this subject. On the other hand a change as small as 1 mg. in the average daily total retention of iron over a shorter period of time may

be considered significant in relation to the intake or excretion. It is not to be expected that iron retention and hemoglobin formation parallel each other for the retention of iron is but one step in its utilization.

A study of the complete metabolic picture for the subjects for the entire period is important for an understanding of their actual iron requirement. Following the current practice of considering the amount of a mineral which is excreted as representing the minimum requirement for that mineral, the minimum total daily iron requirement to cover the complete normal losses during a menstrual cycle for subjects A (omitting the periods of iron therapy), B, C and D, would be 11.9, 11.7, 9.16 and 11.9 mg., respectively. It is important that these minimum intakes would not provide for the replacement of any extra or unusual loss of iron such as a nosebleed or taking venous blood samples. Even though the subjects were receiving approximately only their minimal requirement of iron from the diet they increased the hemoglobin content of their blood slightly more than 1 gm. per 100 cc. This increase represented the utilization of 120 to 140 mg. of iron which had to come from bodily stores. Further increases in hemoglobin values would have been advisable but to what extent they would have been possible without replenishment of bodily stores is a question. Furthermore it is reasonable to assume that the maintenance of a level of iron stores above that of bare minimum is in greatest accord with optimum health. This together with the fact that iron is needed in the body for purposes other than blood building indicates the need of an intake of iron for these subjects which is greater than their minimal requirement. It has been customary to add 50% to a minimal requirement of a dietary essential and designate the result as an optimal allowance. On this basis the optimal allowance for subjects A, B, C and D would be 17.8, 17.5, 14.4 and 17.8 mg. of iron daily.

These results suggest certain facts regarding the iron requirement of normal women. The average daily fecal and urinary excretion of iron for all subjects for the entire time

of the study was 0.193 mg. per kilogram. Considering this figure as the minimal requirement and 50% above this or 0.289 mg. as the optimal allowance, the respective values for a 56 kilo. woman would be 10.8 mg. and 16.2 mg. The question then arises as to whether the optimal allowance will provide for the replacement of iron lost periodically in the menses or whether a separate allowance should be made to cover this loss. This will depend on two factors, the quantity of iron lost in the menses and the length of the menstrual cycle or the time between losses when the body can compensate for the previous loss or store iron in advance of the next one. Using Barer's average figure of 19.5 mg. for the iron content of the menses and assuming a menstrual cycle of 28 days in length it may be calculated that an average daily retention of 0.7 mg. would be necessary to compensate for such a menstrual loss. The dietary standard for a normal 56 kilo. woman would thus approximate 17 mg. per day. Another method of using the results of this study to calculate the daily iron requirement of women is to take the average total loss per kilogram per day of all the subjects, increase it 50% and multiply by the weight of an average woman. Thus 0.206 mg. per kilogram per day which covers losses in the feces, urine, and menses would be increased to 0.309, and then multiplied by 56 to give the total daily optimal allowance of iron which is 17.3 mg. The results obtained by either method of calculation are higher, though in reasonable agreement with the standard of 15 mg. of iron per day recommended by Sherman ('33).

It is a well-known fact that unless particular care is taken in planning a diet it will not contain the recommended 15 mg. of iron per day. The diet in the present study bears evidence to this. It was planned to represent a well-chosen mixed dietary with no emphasis on foods rich in iron and as a result it averaged only 10 to 13 mg. per day.

Parallel with the generally accepted intakes of iron which are lower than optimum are the generally accepted lower hemoglobin values for women. The latter are usually explained as due to the periodic loss of blood in the menses and

therefore were probably unavoidable. Since our present knowledge of the relatively small losses of iron and blood in the menses minimizes this as an etiologic factor it would seem that the low hemoglobin values are more directly a reflection of the prevalence of iron-deficient diets consumed over long periods of time than of excessive losses of iron in the menses. This does not mean that even a small loss should be disregarded when it is recurrent and continues through many years as does the menstrual loss, but neither should it be used to justify low hemoglobin values if they could be corrected by the ingestion of diets optimum in iron content.

SUMMARY AND CONCLUSIONS

The total iron exchanges of four normal young women on an adequate diet during consecutive menstrual cycles were determined over a period of 3 to 5 months by means of a continuous balance experiment.

The total time of the experiment was divided arbitrarily into consecutive 5-day periods and a single foundation dietary was used for every subject during every period. Certain low-mineral foods were permitted ad libitum to meet the differing energy requirements of the subjects. Food composites which represented one-tenth the amount of all food eaten by each subject were made and analyzed for each period.

Feces and urine collections were combined for each 5-day period and analyzed for iron. The menstrual flow was collected on cellulose pads for analysis. Records were kept of any additional blood losses and the results considered in the actual iron balance of the subjects.

The average daily intakes of iron for the entire time for the four subjects were 13.61, 11.87, 10.03 and 11.71 mg. with corresponding balances between food and excreta of + 0.72, + 1.48, + 0.71 and — 0.20 mg. The average coefficient of variation was 11.9 for the iron in the food and 25.8 for that in the excreta.

Although there were periods of negative and of positive balance for the subjects during the study, a negative balance never occurred when the intake of iron from food was 0.225

mg. or more per kilogram per day. No relation between time of iron storage and its loss during the menstrual period could be demonstrated.

A supplement of 5 mg. of iron in the form of ferric ammonium citrate was given to one subject during her fourth and fifth menstrual cycles. This resulted in an increased excretion and a decreased retention of iron as compared with the cycles when she was not receiving the supplement.

The average menstrual losses for the four subjects were 14.26, 22.84, 11.13 and 13.80 mg. of iron, respectively. The losses were relatively constant from period to period for the same subject.

In nine of the sixteen menstrual cycles the subjects were in iron equilibrium or slight positive balance after the menstrual loss had been subtracted from the balance between the iron intake and iron excretion for each cycle.

Only two subjects were able to compensate also for the iron loss in the venous blood samples which were taken in connection with another study. The other two subjects were in negative iron balance when this additional loss was included in calculating their actual iron balance for the entire study.

All the subjects increased the hemoglobin content of their blood at least 1 gm. per 100 cc. during the experimental regime; the red cell content remained remarkably constant.

The findings are discussed in relation to the iron requirement of these subjects and of other normal women. These subjects on a diet which contained 10 to 14 mg. of iron daily and fortified in other dietary essentials were receiving only their minimal requirement of this element. Calculations for the optimal daily allowance of iron for a 56 kilo. woman give results of 16 to 17 mg. which is somewhat higher than the 15 mg. recommended by Sherman. The results of this study together with the fact that the average dietary seldom contains even 15 mg. of iron indicate that the low hemoglobin values which are accepted as normal for women because of the drain due to menstruation may be a direct reflection of the use of diets habitually low in iron rather than due to the small periodic loss in the menses.

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THE UTILIZATION OF ENERGY PRODUCING NUTRIMENT AND PROTEIN AS AFFECTED BY SODIUM DEFICIENCY [1]

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ONE FIGURE

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This study was undertaken as a unit in a series of investigations in the light of the conception that any deficiency in any essential nutrient must eventually affect food utilization in general, the specific objects of these studies being to demonstrate the ways in which, and the degrees to which individual nutritive deficiencies affect the utilization of food energy and protein.

It is natural to think of sodium, in animal nutrition, in association with chlorine, since these two elements occur in diets predominately in combination as sodium chloride; and, in regard to the functions of this salt in nutrition, the attention naturally concerns itself mainly with the chlorine, in its obviously important relation as a constituent of the gastric juice—the accompanying sodium being considered mainly in connection with its own utilization, and with the process ('alkaline tide') of its elimination from the body.

In the present study, however, chlorine and the metabolism of sodium in combination with chlorine, are eliminated from the nutritional picture, by the provision of chlorine, in abundance, to all experimental subjects, thus turning the light of inquiry upon the element sodium.

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In harmony with the extreme solubility of the salts of sodium, and with the fact of the marine origin of terrestrial animal life, sodium is omnipresent within the body, but is most abundant in the extracellular fluids. It contributes to the normal electrolyte contents of the body, and to the maintenance of acid-base balance, osmotic equilibrium, muscular irritability, and control of heart action. Its functions, therefore, are broad and fundamental.

The literature on the functions of sodium has been reviewed by Barker, Hoskins and Mosenthal ('22), Peters and Van Slyke ('31), Heubner ('31), Klinke ('31), Meyer-Bisch ('31), Sherman ('32) and Bodansky ('34).

A number of determinations have been made of the sodium requirement of the rat. Thus, Osborne and Mendel ('18) found that rats 'grew with vigor' on a diet containing 0.035% of sodium, and that "less than 0.04% of either sodium or chlorine in the food sufficed to permit these rats to complete their growth." H. G. Miller ('23) obtained satisfactory growth of rats on a synthetic diet containing 0.07% of sodium. Later Miller ('26) failed to get growth from a diet containing 0.03% of sodium, but did obtain satisfactory growth when the sodium content of the diet was increased to 0.42% sodium (our calculation). Olson and St. John ('25) found 0.23% of sodium in the diet of the rat to be insufficient, but found 0.53% to be satisfactory for growth and reproduction. Mitchell and Carman ('26) concluded, from growth and metabolism experiments with rats and chickens, that "the addition of sodium chloride to a ration composed largely of corn, containing 0.047% sodium and 0.041% chlorine, enhances its growth-promoting value." Schoorl ('34) found that on a diet containing 0.009% sodium the growth of rats was limited to the attainment of a body weight of about 60 gm. during 10 weeks, and that rats made a good growth on a diet with a sodium percentage of 0.2. Richards, Godden and Husband ('24) found that the addition of sodium chloride or citrate to a ration of cereal grain fed to a growing pig led to increased assimilation and retention of nitrogen, calcium and phosphorus.

Also Sjollema ('35) found the growth of chicks considerably restricted by rations poor in sodium, especially when containing no more than 0.013% of this element.

That a greater degree of concordance was not found in the foregoing observations of sodium requirements of rats was doubtless due to the facts that the influence of the accompanying chlorine intake was not in all cases excluded, and that the sodium intake and the plane of nutrition differed, as also did the judgment of the experimenters as to what constituted satisfactory growth.

No reports have come to the attention of the authors of investigations in which the relation of sodium alone to efficiency of food utilization has been studied in extensive and rigidly controlled experiments of long duration. With such considerations, therefore, the present study is concerned.

EXPERIMENTAL PROCEDURE

A sodium deficient and a sodium supplemented diet were compared by means of a 10-week growth, metabolism and body analysis study, with twelve pairs of weanling albino rats as subjects, each pair being of one sex and the same litter, with feed intake controlled by the paired method. The details of technic were the same as described in a recent paper by Swift, Kahlenberg, Voris and Forbes ('34).

The composition of the two diets was as follows:

Na-deficient diet		*Na-supplemented diet*	
Ground corn (maize)	78.90	Ground corn (maize)	78.90
Casein (vitamin-free)	14.00	Casein (vitamin-free)	14.00
Cod liver oil	2.00	Cod liver oil	2.00
Calcium chloride ($2H_2O$)	0.10	Calcium chloride ($2H_2O$)	0.10
Dextrin	4.00	Dextrin	1.05
Calcium carbonate	1.00	Calcium carbonate	1.00
Sodium bicarbonate	0.00	Sodium bicarbonate	1.75
Crisco	0.00	Crisco	1.20

The two diets were maintained equicaloric by adjustment of their contents of dextrin and of hydrogenated vegetable oil.[3] By analysis the supplemented diet contained 0.502%, and the deficient diet 0.007%, of sodium.

[3] Crisco.

In order to prevent an extensive storage of sodium during the preliminary growth interval the young rats and their mothers were given the sodium deficient diet from the sixteenth day after the birth of the young until the beginning of the experiment.

At the end of the experiment, the rats were killed, and the bodies were analyzed for moisture, ether extract, nitrogen and energy, as in the other experiments of this series, the heat production being computed by the body balance procedure, that is, as the difference between the gross energy of the food and the sum of the energy of the body increase and the excreta. In addition the sodium content of the rat bodies was determined.

Sodium was determined in the diets and in the rat bodies essentially by Butler and Tuthill's ('31) application of the method of Barber and Kolthoff ('28).

PRESENTATION OF RESULTS

In consideration of the complexity and the fundamental nature of the functions of sodium in nutrition it seems inevitable that the effects of sodium deficiency should be general in character; and thus they were found to be—that is, sodium deficiency was apparent especially as it affected nutrition in general, rather than in an obviously specific way.

Among the entire series of nutritive deficiency studies that have been made in this laboratory the present experiment was unique in the promptness, the decisiveness and the consistency with which the deficiency, in this case of sodium, served to diminish the rate of growth.

In this experiment there were 396 refusals of feed, 308 by the animals on the sodium deficient diet, and eighty-eight by the rats on the supplemented diet. The sodium deficient rats, therefore, restricted the food intake of their pair mates.

In relation to the quantitative results—all average data as presented represent twelve animals on continuous metabolism investigation during a 10-week period.

All the rats which received the sodium supplemented diet made greater growth than did their pair mates (fig. 1). The average gains in body weight of the rats, with contents of alimentary tract removed, on the sodium deficient and sodium supplemented diets were 31.47 gm. and 63.29 gm., respectively, the dry matter of the food eaten being 373.5 and 374.3 gm., respectively, while the dry matter of food per gram of body increase was 11.9 ± 0.26 gm. for the rats on the deficient diet, and 5.9 ± 0.22 gm. for the rats which received the sodium supplemented diet.

TABLE 1

Gain in body weight[1] of rats as related to dry matter of food

PAIR	Na-DEFICIENT DIET			Na-SUPPLEMENTED DIET		
	Food eaten (dry matter)	Gain in body weight	Dry matter of food per gram body gain	Food eaten (dry matter)	Gain in body weight	Dry matter of food per gram body gain
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	434.7	36.48	11.9	435.7	101.45	4.3
2	382.0	25.62	14.9	382.9	77.70	4.9
3	379.9	35.57	10.7	380.7	76.59	5.0
4	371.7	32.69	11.4	372.5	50.51	7.4
5	409.7	38.61	10.6	410.6	68.81	6.0
6	349.6	30.23	11.6	350.4	53.41	6.6
7	428.2	40.10	10.7	429.2	74.52	5.8
8	358.2	29.94	12.0	359.0	63.31	5.7
9	360.6	27.63	13.1	361.4	57.56	6.3
10	346.6	25.94	13.4	347.4	45.56	7.6
11	357.2	32.17	11.1	358.0	51.36	7.0
12	303.0	22.60	13.4	303.7	38.67	7.9
Average	373.5	31.47	11.9	374.3	63.29	5.9

[1] Excluding contents of alimentary tract.

Since the energy intake was the same with both groups, the greater gain of body weight by the rats on the sodium supplemented diet was necessarily made from a smaller proportionate food expenditure.

The digestibility of the diets was unaffected by the difference in sodium content, the average coefficients for nitrogen being 91.5± 0.20 and 92.4 ± 0.17%, and for energy 92.1 ± 0.08 and 92.4 ± 0.06%, respectively, for the sodium deficient and the sodium supplemented diets.

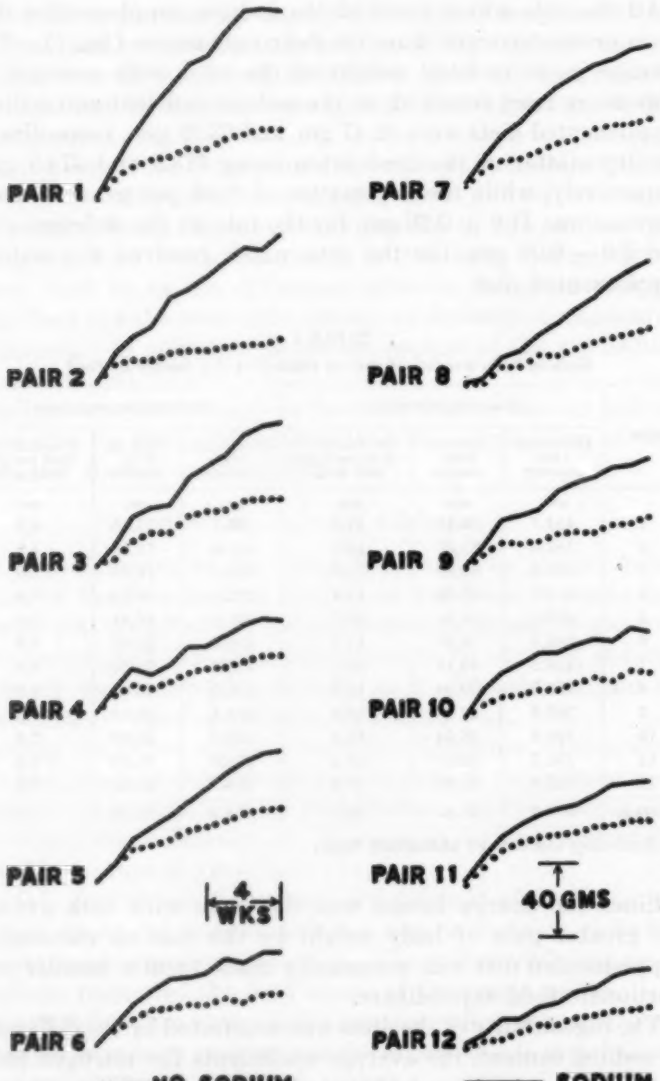

Fig. 1 The growth of albino rats, during 10 weeks, as affected by sodium deficiency.

In regard to energy storage there was a remarkable difference in favor of the sodium supplemented diet, the average body gains being 63.0 and 133.9 Calories, which were equivalent to $3.5 \pm 0.15\%$ and $7.5 \pm 0.32\%$ of the food energy, for the sodium deficient and the sodium supplemented rats, respectively.

TABLE 2

Energy of body gain of rats as related to energy and to metabolizable energy of food

PAIR	FEED ENERGY	Na-DEFICIENT DIET				Na-SUPPLEMENTED DIET			
		Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy	Body gain		Metabolizable energy	Body gain as percentage of metabolizable energy
			Per cent of feed energy				Per cent of feed energy		
	Cal.	*Cal.*			%	*Cal.*			%
1	2074.9	66.5	3.2	1803.5	3.7	178.1	8.6	1830.7	9.7
2	1823.1	51.9	2.8	1601.2	3.2	167.2	9.2	1615.3	10.4
3	1813.0	64.3	3.5	1598.5	4.0	142.3	7.8	1610.5	8.8
4	1774.1	82.0	4.6	1567.8	5.2	96.6	5.4	1562.2	6.2
5	1955.2	100.4	5.1	1721.7	5.8	194.3	9.9	1728.4	11.2
6	1668.7	52.8	3.2	1475.9	3.6	121.6	7.3	1476.0	8.2
7	2043.6	82.1	4.0	1804.4	4.5	185.8	9.1	1787.2	10.4
8	1709.7	64.9	3.8	1516.2	4.3	121.0	7.1	1511.6	8.0
9	1721.2	59.1	3.4	1517.0	3.9	140.7	8.2	1522.7	9.2
10	1654.3	45.3	2.7	1469.7	3.1	72.4	4.4	1455.2	5.0
11	1704.6	53.3	3.1	1498.9	3.6	98.8	5.8	1492.9	6.6
12	1446.1	33.0	2.3	1274.3	2.6	87.5	6.1	1280.0	6.8
Aver.	1782.4	63.0	3.5	1570.8	4.0	133.9	7.5	1572.7	8.5

The metabolizable energy of the diets was essentially the same, being 1570.8 and 1572.7 Calories, respectively, the body gains of metabolizable energy being 4.0 ± 0.17 and $8.5 \pm 0.36\%$, respectively, for the sodium deficient and the sodium supplemented diets.

The rats on the sodium supplemented diet also materially exceeded their pair mates on the deficient diet in regard to nitrogen retention. On an average the gains of nitrogen were 1.40 and 2.46 gm., which were equivalent to 10.5% and 18.5%, respectively, of the food nitrogen, for the sodium deficient and the sodium supplemented rats.

The character of the total gains in energy is indicated by the facts that of the 63.0 Calories gained by the sodium deficient rats, 47.3 Calories was in the form of protein and 15.7 Calories in the form of fat, while of the 133.9 Calories gained by the sodium supplemented rats 84.9 Calories was in the form of protein and 49.0 Calories in the form of fat.

TABLE 3

Nitrogen of body gain related to fat and energy of body gain and to nitrogen of feed

PAIR NO.	NITROGEN OF BODY GAIN	FAT GAINED		ENERGY GAINED			NITROGEN OF FEED	
			Per gram nitrogen gained	Total	As protein	As fat		Utilized for body gain
Sodium deficient diet								
	gm.	*gm.*	*gm.*	*Cal.*	*Cal.*	*Cal.*	*gm.*	*%*
1	1.52	1.5	1.0	66.5	53.1	13.4	15.49	9.8
2	1.12	1.4	1.3	51.9	39.0	12.9	13.61	8.2
3	1.55	1.2	0.8	64.3	52.6	11.7	13.54	11.4
4	1.46	3.5	2.4	82.0	48.8	33.2	13.25	11.0
5	1.63	4.9	3.0	100.4	54.1	46.3	14.60	11.2
6	1.38	0.8	0.6	52.8	45.2	7.6	12.46	11.1
7	1.72	2.3	1.3	82.1	60.1	22.0	15.26	11.3
8	1.30	2.0	1.5	64.9	45.8	19.1	12.77	10.2
9	1.19	2.0	1.7	59.1	40.5	18.6	12.85	9.3
10	1.24	0.3	0.2	45.3	42.1	3.2	12.35	10.0
11	1.51	0.5	0.3	53.3	48.6	4.7	12.73	11.9
12	1.14	−0.5	−0.4	33.0	37.7	−4.7	10.80	10.6
Average	1.40	1.7	1.1	63.0	47.3	15.7	13.31	10.5
Sodium supplemented diet								
1	3.94	4.6	1.2	178.1	136.3	41.8	15.49	25.4
2	2.90	7.2	2.5	167.2	101.1	66.1	13.61	21.3
3	2.93	4.5	1.5	142.3	100.1	42.2	13.54	21.6
4	2.07	2.7	1.3	96.6	71.1	25.5	13.25	15.6
5	2.57	11.5	4.5	194.3	87.1	107.2	14.60	17.6
6	2.01	5.4	2.7	121.6	70.9	50.7	12.46	16.1
7	2.74	9.8	3.6	185.8	93.5	92.3	15.26	18.0
8	2.44	3.7	1.5	121.0	85.6	35.4	12.77	19.1
9	2.15	7.0	3.3	140.7	75.5	65.2	12.85	16.7
10	1.99	0.6	0.3	72.4	66.6	5.8	12.35	16.1
11	2.10	2.8	1.3	98.8	72.1	26.7	12.73	16.5
12	1.66	3.1	1.9	87.5	58.4	29.1	10.80	15.4
Average	2.46	5.2	2.1	133.9	84.9	49.0	13.31	18.5

The heat loss for the rats on the sodium deficient and sodium supplemented diets was 1507.8 Calories and 1438.9 Calories, or 84.6 ± 0.17 and 80.7 ± 0.31% of the food energy, respectively. The statistical significance of this difference is expressed by odds of more than 10,000 to 1.

The method of this investigation does not reveal the relative extent to which the observed difference in heat produc-

TABLE 4

Digestibility of nitrogen and energy producing nutriment

PAIR	Na-DEFICIENT DIET				Na-SUPPLEMENTED DIET			
	Nitrogen		Energy		Nitrogen		Energy	
	Feces	Digested	Feces	Digested	Feces	Digested	Feces	Digested
	gm	%	*Cal.*	%	*gm.*	%	*Cal.*	%
1	1.78	88.5	183.6	91.2	1.40	91.0	157.2	92.4
2	1.15	91.6	151.6	91.7	1.03	92.4	136.2	92.5
3	1.14	91.6	141.3	92.2	0.96	92.9	129.0	92.9
4	1.06	92.0	135.4	92.4	0.96	92.8	131.7	92.6
5	1.14	92.2	155.0	92.1	0.97	93.4	148.1	92.4
6	0.93	92.5	125.5	92.5	0.83	93.3	121.8	92.7
7	1.30	91.5	161.6	92.1	1.33	91.3	169.5	91.7
8	0.94	92.6	127.3	92.6	0.88	93.1	129.4	92.4
9	1.06	91.8	137.9	92.0	0.97	92.5	128.9	92.5
10	1.02	91.7	120.8	92.7	1.08	91.3	129.1	92.2
11	1.17	90.8	136.1	92.0	1.04	91.8	131.9	92.3
12	0.85	92.1	114.5	92.1	0.67	93.8	103.9	92.8
Average	1.13	91.5	140.9	92.1	1.01	92.4	134.7	92.4

tion is to be accounted for as maintenance quota and as energy expense of food utilization.

The markedly different sodium intake in the two diets was reflected in a moderate difference in the percentage of sodium in their bodies (0.325 ± 0.004, as compared with 0.368 ± 0.002), in favor of the rats on the sodium supplemented diet, and a decided difference, also in favor of the rats on the sodium supplemented diet, in the average quantity of sodium in their bodies (0.072 ± 0.001 gm., as compared with 0.114 ± 0.003 gm.).

The moisture content of the bodies of the rats did not differ significantly, as influenced by the diets, the average value for the sodium deficient rats being $67.6 \pm 0.28\%$, and for the sodium supplemented rats $66.9 \pm 0.35\%$.

TABLE 5

Heat loss related to energy of food

PAIR	FEED ENERGY	Na-DEFICIENT DIET		Na-SUPPLEMENTED DIET	
		Heat loss		Heat loss	
			Per cent of feed energy		Per cent of feed energy
	Cal.	*Cal.*		*Cal.*	
1	2074.9	1737.0	83.7	1652.6	79.6
2	1823.1	1549.3	85.0	1448.1	79.4
3	1813.0	1534.2	84.6	1468.2	81.0
4	1774.1	1485.8	83.7	1465.6	82.6
5	1955.2	1621.3	82.9	1534.1	78.5
6	1668.7	1423.1	85.3	1354.4	81.2
7	2043.6	1722.3	84.3	1601.4	78.4
8	1709.7	1451.3	84.9	1390.6	81.3
9	1721.2	1457.9	84.7	1382.0	80.3
10	1654.3	1424.4	86.1	1382.8	83.6
11	1704.6	1445.6	84.8	1394.1	81.8
12	1446.1	1241.3	85.8	1192.5	82.5
Average	1782.4	1507.8	84.6	1438.9	80.7

TABLE 6

The sodium content of rat bodies

PAIR NO.	SEX	SODIUM DEFICIENT DIET	SODIUM SUPPLEMENTED DIET
		gm.	*gm.*
1	M	0.070	0.157
2	M	0.073	0.129
3	M	0.081	0.129
4	M	0.076	0.109
5	F	0.074	0.115
6	F	0.068	0.100
7	F	0.080	0.122
8	M	0.068	0.108
9	F	0.064	0.100
10	F	0.069	0.098
11	M	0.076	0.101
12	F	0.065	0.094
Average		0.072	0.114

After 4 or 5 weeks on the experiment, the sodium deficient animals manifested a craving for sodium by licking and biting the fingers of the attendant, when being handled for weighing; and after 8 weeks on experiment these rats evinced an increased desire for salt by licking the sides of the cage and water bottle immediately after feeding.

At no time during the 10-week experiment, however, did the sodium deficient rats exhibit ocular or visual abnormality, or such a decline in weight as was reported by St. John ('28).

SUMMARY

In a growth, metabolism, and body analysis experiment, by the paired feeding method, sodium deficiency unfavorably affected the appetite, the increase in weight, the storage of energy and the synthesis of fat and protein—the proportion of the energy stored as fat being the larger with a sodium supplemented than with a sodium deficient diet.

There were no significant differences in the digestibility of the protein, or in the digestibility or the metabolizability of the energy producing nutrients, of the two diets, or in the moisture contents of the bodies of the two groups of rats.

The heat loss was significantly higher with the rats on the sodium deficient than with those on the sodium supplemented diet.

Decidedly more sodium was stored in the rat bodies from the sodium supplemented than from the sodium deficient diet, with resulting higher percentage of sodium in the bodies of the rats of the former group.

With food intake restricted by their pair mates on a diet containing 0.007% sodium, rats on a diet containing 0.502% sodium increased in body weight from 51 gm. to 116 gm. during 70 days, that is, at a rate of 0.9 gm. per day, while a diet containing 0.007% sodium permitted growth at only about half the rate stated.

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THE UTILIZATION OF HEXOSES BY EXCISED RAT TISSUES [1]

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ONE FIGURE

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In the preparation of artificial media for tissue metabolism studies the assumption frequently has been that glucose, in a concentration equal to or greater than that of the blood was necessary for a more normal value of the metabolism and to maintain it for any considerable period of time. Evidence has been accumulating which indicates that in certain tissues added glucose is not utilized—at least that it is not burned.

With liver tissue Crabtree ('29), Quastel and Wheatley ('33) and Kisch ('33, '34) measuring only oxygen consumption found no increase with glucose. Dickens and Greville ('33), Himwich et al. ('34) and Elliott and Baker ('35) found no change in the R.Q. as well.

With kidney tissue there are conflicting reports on the effect of glucose. Shorr, Loebel and Richardson ('30) in addition to some of the above workers, found increases in both O_2 and R.Q. when glucose was added, but Kisch found no difference in the O_2 uptake whereas Himwich obtained a rise in O_2 but only an insignificant rise in R.Q.

Richardson, Shorr and Loebel ('30) found no difference in either O_2 or R.Q. of muscle strips with and without glucose and Kisch, using rat diaphragm found no change in O_2 uptake with any of the three hexoses.

[1] This investigation was assisted by a grant from the Corn Industries Research Foundation.

Many of the other tissues have been found to oxidize added glucose to a considerable extent but liver and muscle seem to stand out as two which do not, although there has been but little work done with muscle. The evidence for the oxidation of glucose by kidney is conflicting.

Investigations on fructose and galactose have yielded the following results. With liver, Dickens and Greville found that fructose produced a rise in R.Q. but none in O_2 consumption, while Kisch found a slight rise in O_2 with fructose but not with galactose. The latter also found no effect of galactose on kidney but an increase with fructose, in accord with the results of Dickens and Greville.

The present research was limited to a study of the influence of the three hexoses, glucose, fructose and galactose, upon the respiratory exchange of liver, kidney and muscle tissue. In many of the groups both fed and fasted rats were employed to determine whether or not the nutritive condition of the tissues played a role in their utilization of the sugar and whether or not this circumstance might explain some of the conflicting reports in the literature.

METHOD

The studies were carried out by means of the Warburg apparatus with a modified bottle in which measurements of both O_2 and CO_2 were made on the same tissue slices. The bottle, shown in figure 1, contains a central partition high enough to permit the bottles to be shaken with as much as 3 cc. of fluid in each half without danger of its spilling over. The tissue in Ringer's-phosphate solution is placed in one side and N/5 $Ba(OH)_2$ in the other side. A side arm was added to each half of the bottle to hold N/2 HCl. This permits the tissue and medium, as well as the alkali, to be treated with acid for the release of any CO_2 which may have been held in either. A correction is made for the amount of CO_2 in the tissue and fluid at the beginning, determined on a separate sample of tissue.

While this method still necessitates the measurement of CO_2 and O_2 in a CO_2 free atmosphere, and in a medium buffered with phosphate alone, both of which are unphysiological, it does eliminate the error which may be involved in the original Warburg method of measuring CO_2 production and O_2 absorption on different samples of tissue. Serum, however, may be used in place of the Ringer's-phosphate solution with results only slightly better for ordinary conditions of measurement at 37.5°C. (Marsh, '34).

Fig. 1 A new bottle for the Warburg apparatus, having two chambers each with side sack, permitting determination of preformed CO_2, O_2 and final CO_2.

Simultaneous determinations in triplicate were made of the oxygen consumption and of the respiratory quotient with and without the various sugars. Measurements were made for 1 hour at 37.5°C. and O_2 values are expressed in mm.^{3} per hour per milligram of dry weight of tissue. Triplicate values which varied by more than 7 to 8% were discarded. R.Q.'s seldom varied more than 0.05 and usually not more than 0.02 or 0.03. In some of the experiments on liver the determinations were made in air (as indicated in the tables) but all

others were made in oxygen. The glucose used was a pure product supplied by Corn Industries Research Foundation and the fructose and galactose were obtained from Pfanstiehl Chemical Company.

The medium was the usual Ringer's solution buffered with N/150 phosphate mixture of pH 7.4 (Warburg medium described by Richardson, '29) and the sugar solutions were made up in this medium to a final concentration of 0.2%. It was found after the experiments were practically completed, that the pH of the Ringer's-phosphate solution, as determined by the quinhydrone method, was not 7.4 but about 7.1. However, check experiments at both reactions showed that, although the O_2 values for liver were slightly higher at pH 7.4 than at 7.1, the response to the sugars was the same and with kidney tissue there was no difference, even in the absolute values.

The finding of Quastel and Wheatley ('34) also was confirmed that M/150 phosphate buffer is not strong enough to maintain the pH of the solution in which liver is suspended. The change may be from 0.3 to 0.6 pH in an hour. More concentrated solutions of phosphate precipitate calcium ions and glycerophosphate was found to be but little better in maintaining the original pH. With kidney tissue, there is little or no drop in the pH of the Ringer's-phosphate solution during an hour's determination.

RESULTS

The mean values for the oxygen consumption and the R.Q. of kidney tissue obtained with all three sugars are summarized in tables 1 and 2. Data are treated statistically with the standard deviation, the probable error of the mean and coefficient of variability in table 1 for both fed and fasted rats. Table 2 gives the difference of the means, the probable error of the difference and the ratio of these two values, which should be over 3 if the difference between the mean values is significant.

TABLE 1

Oxygen consumption and respiratory quotients of kidney tissues with and without sugar

MEDIUM	NUMBER OF EXPERIMENTS	O_2-MM.^{3}/HR./MG.					R.Q.				
		Mean	Increase	S.D.	P.E. of mean	V	Mean	Theoretical R.Q.	S.D.	P.E. of mean	V
Fed rats											
			per cent			*per cent*					*per cent*
No sugar	11	22.68		1.67	±0.24	7.4	0.767		0.032	±0.005	4.2
Glucose	11	29.41	27	1.60	±0.25	5.5	0.783	0.820	0.029	±0.004	3.7
Fructose	11	34.04	50	2.77	±0.42	8.1	0.846	0.849	0.023	±0.003	2.7
No sugar	7	24.19		2.10	±0.38	8.6	0.762		0.016	±0.003	2.1
Galactose	7	24.53	1	2.13	±0.38	8.7	0.761		0.011	±0.002	1.4
Rats fasted 24 to 48 hours											
No sugar	10	25.86		1.40	±0.21	5.5	0.748		0.029	±0.004	3.8
Glucose	10	30.42	18	1.65	±0.24	5.4	0.746	0.786	0.022	±0.003	2.9
Fructose	10	34.66	33	3.50	±0.55	10.1	0.813	0.811	0.024	±0.004	3.0

S.D. = standard deviation. P.E. = probable error. V = coefficient of variability.

TABLE 2

Difference of the means, the P.E. of the difference of the means and the ratio of the two calculated for the data on kidney in table 1

COMPARISON OF	O_2-MM.^{3}/HR./MG.			R.Q.		
	Difference of means	P.E. of difference of means	Difference of means / P.E. of difference	Difference of means	P.E. of difference of means	Difference of means / P.E. of difference
Fed rats						
No sugar—glucose	6.33	±0.35	18.1	0.016	±0.006	2.6
No sugar—fructose	11.36	±0.48	23.7	0.079	±0.006	13.1
No sugar—galactose	0.34	±0.54	0.6	0.001	±0.008	0.1
Fasted rats						
No sugar—glucose	4.56	±0.32	14.2	0.002	±0.005	0.4
No sugar—fructose	8.80	±0.59	14.9	0.065	±0.004	16.2

It will be noted that glucose produced a rise in the O_2 uptake of 27% in the kidney of fed and 18% in that of fasted animals. Such increases are significant since the ratios are 18 and 14, respectively. In contrast the R.Q. with glucose rose not at all in the fasted group and an insignificant amount in the fed group and is definitely below the theoretical R.Q. calculated on the assumption that all the extra O_2 was occasioned by the combustion of the sugar. The fact that the coefficients of variability were always lower for the R.Q. values than for the O_2 values helps to establish the accuracy of the observation that glucose does not produce a rise in R.Q. with kidney tissue in spite of a significant rise in O_2 uptake.

Fructose produced a still greater rise in O_2 consumption, viz., 50% and 33% in the fed and fasted rats, respectively, with ratios of significance of 24 and 15 in the two groups. In this case the R.Q. rose also, sufficiently to give ratios of 13 and 16 and to be within 0.002 and 0.003 of the theoretical R.Q.

Seven experiments with and without galactose showed no difference in either the O_2 uptake or the R.Q., the ratios being 0.6 for the O_2 and 0.1 for the R.Q. Only fed animals were used for this study, since there was no striking difference in the response between fed and fasted animals to the other two sugars and the little difference observed being in favor of the fed animal.

Attention might be called to the absolute values of the O_2 consumption of kidney slices measured in oxygen without sugar. A mean value of 22.7 mm.^{3} for one series and of 24.2 for another shorter series is considerably higher than many of the values reported in the literature, including a previous one from this laboratory (Marsh, '34). In our own work, thinner slices were responsible for the higher values and slices too thick to permit adequate diffusion of O_2 may afford the explanation for other low values. Since the Q_{O_2} of the kidney is two to three times that of liver and most of the other tissues, the slices should be correspondingly thinner.

The results on liver are summarized in tables 3 and 4. In several of the groups, values obtained in air are averaged with those obtained in oxygen since the R.Q. and the response to the sugars were the same in both. Such mixed groups do not lend themselves to statistical treatment, however, because

TABLE 3

Oxygen consumption and respiratory quotient of liver tissue with and without sugars

MEDIUM	NUMBER OF EXPERIMENTS	O_2-MM.^{3}/HR./MG.			R.Q.		
		Mean	Difference	Difference	Mean	Difference	Theoretical R.Q.
Fed rats							
				per cent			
No sugar	17[1]	8.56			0.721		
Glucose		8.26	—0.30	—4.6	0.720	—0.001	
No sugar	9[2]	8.19			0.722		
Fructose		8.82	+0.63	+7.7	0.814	+0.092	0.741
Glucose	14[3]	5.27			0.737		
Fructose		5.80	+0.53	+10.1	0.819	+0.082	0.760
Galactose	11[4]	7.18			0.727		
Fructose		8.30	+1.12	+15.6	0.830	+0.103	0.764
Fasted rats							
No sugar	10[5]	6.36			0.593		
Glucose		6.55	+0.19	+3.0	0.595	+0.002	
No sugar	10[6]	6.22			0.597		
Fructose		7.35	+1.13	+18.0	0.726	+0.129	0.659
Glucose	5[7]	4.86			0.597		
Fructose		5.45	+0.59	+12.1	0.725	+0.128	0.630
Galactose	11[8]	5.16			0.601		
Fructose		5.51	+0.35	+6.8	0.735	+0.134	0.626

[1] Three in air. [2] Two in air. [3] All in air. [4] Four in air.
[5] Five in air. [6] Five in air. [7] All in air. [8] All in air.

the absolute values for the oxygen absorption were definitely lower in air than in oxygen. This would mean a high standard deviation and a correspondingly low ratio of significance, even in groups in which the percentage increase with sugar is undoubtedly significant (15 and 19% in two instances). Table 4 gives the probable errors, the standard deviations and

significance ratios for only those groups in which the experiments were either all done in air or all done in oxygen.

With neither the fed nor fasted liver did glucose produce any effect. There was no rise in the oxygen uptake or in the R.Q. Oxygen absorption with fructose, when compared to that obtained without sugar, with glucose and with galactose was, in each case, higher (table 3). A larger number of experiments would undoubtedly make the percentage increase

TABLE 4

Standard deviation, probable error and ratio of significance of difference of the means to the probable error of the difference calculated for parts of the data on liver given in table 3

MEDIUM	O_2 MM.^{3}/HR./MG.			R.Q.		
	Mean	S.D.	Diff. of means / P.E. of diff.	Mean	S.D.	Diff. of means / P.E. of diff.
Fed rats						
No sugar[1]	8.84±0.11	0.42	0.58 / ±0.18 = 3.2	0.731±0.008	0.030	0.085 / ±0.015 = 5.6
Fructose	9.42±0.15	0.60		0.816±0.013	0.051	
Glucose	5.27±0.08	0.47	0.53 / ±0.11 = 4.8	0.737±0.008	0.047	0.78 / ±0.010 = 7.8
Fructose	5.80±0.08	0.47		0.819±0.006	0.032	
Fasted rats						
Glucose	4.86±0.10	0.32	0.59 / ±0.13 = 4.5	0.597±0.008	0.032	0.128 / ±0.010 = 12.8
Fructose	5.45±0.08	0.27		0.725±0.006	0.022	
Galactose	5.16±0.10	0.15	0.35 / ±0.15 = 2.3	0.601±0.009	0.048	0.134 / ±0.011 = 12.2
Fructose	5.51±0.12	0.58		0.735±0.007	0.037	

[1] Seven of the nine experiments listed in table 3, which were done in O_2. All other groups were done in air.

more uniform, since it is evident, particularly from a consideration of the R.Q.'s that liver burned neither the glucose nor the galactose.

There is no consistent difference between the fed and fasted groups, the average rise in O_2 with fructose being 11% in the fed series and 12% in the fasted.

For the groups treated statistically, the ratio of the difference of the means to the probable error of the difference is, for the O_2 absorption, under 5 in each case. Since a ratio of 3

is not a very rigid requirement for measuring the significant difference of the two groups of observations, the rise in O_2 with fructose might be questioned. However, the fact that, in fifty-four out of sixty experiments on both fed and fasted animals, there was a higher O_2 uptake with fructose, makes it seem quite certain that the sugar does produce a definite though small rise in O_2 consumption.

With liver, fructose always caused the R.Q. to rise considerably above the theoretical value for the increase in O_2 uptake, from 0.06 to 0.07 higher in the fed and from 0.07 to 0.11 in the fasted series. The ratio of significance is fairly high and higher in the fasted series.

The low R.Q.'s of liver are of interest and are very consistent in all the groups. The average without sugar is from 0.72 to 0.74 for the fed animals and from 0.59 to 0.60 for the fasted.

Diaphragm was chosen as representative of skeletal muscle since the whole muscle can be used, little injury is involved and it respires at a very uniform rate for several hours. Young rats were used to make certain that the diaphragm was thin enough to allow adequate O_2 diffusion. Two types of experiment were employed. The usual one, involving three bottles without sugar and three with, did not give entirely satisfactory results since with whole diaphragms from different rats, even though litter mates were chosen, the triplicate determinations in many instances, varied over rather a wide range. However, thirty-two experiments of this type (table 5) averaged 5.48 and 5.60 for the O_2 uptake with and without glucose and 0.871 and 0.840 for the R.Q., the rise in the R.Q. being within the error of the method. With fructose the thirteen experiments of this type are too few, considering the wide variation in the triplicate determinations, from which to draw any conclusion, but the number is sufficient to show that there was no trend toward an increase in either the O_2 or the R.Q.

However, the second type of experiment, which proved more satisfactory, gave results on O_2 uptake in accordance with

those obtainable by the usual method. This consisted of placing a solution of the sugars in the side arm above the tissue and measuring the O_2 uptake for an hour (readings every 20 minutes) in Ringer's-phosphate solution, then pouring in the sugar and following the respiration another hour. Obviously it was not possible to make R.Q. determinations under the two conditions with this method, but the mean O_2 values for fifteen such experiments with glucose and twenty-one with fructose showed, in agreement with the findings by the first method, that there was no acceleration of the O_2 absorption with either glucose or fructose.

TABLE 5

Effect of glucose and fructose on oxygen consumption and R.Q. of muscle. Diaphragm of young fed rats

MEDIUM	NUMBER OF EXPERIMENTS	O_2-MM.^{3}/HR./MG.		R.Q.	
		Mean	Difference	Mean	Difference
			per cent		
No sugar	32	5.60		0.840	
Glucose		5.48	—2.1	0.871	+0.031
No sugar	13	6.64		0.897	
Fructose		6.56	—1.2	0.890	—0.007

DISCUSSION

The data presented in this paper, together with that found in the literature, furnish definite proof of the striking differences in the ability of the tissues to oxidize monosaccharides in vitro. One would expect that the sugar of the blood would be easily and universally oxidized. Such is not the case. Of the three tissues studied, only kidney has its respiratory metabolism altered by glucose in the surrounding medium. In these experiments, in agreement with those of Himwich et al. ('34) the effect seems to be more in the nature of a stimulation of the metabolism than a direct oxidation of the sugar since it was only the O_2 absorption which was increased. Other observers (Shorr, Loebel and Richardson, '30; Dickens and Greville, '33; and Ellicott and Baker, '35) found evidence of a direct oxidation with a rise in R.Q. as well as in O_2 up-

take. The reason for this difference between our results and theirs is not apparent.

With liver and muscle, two tissues actively concerned with carbohydrate metabolism in vivo, no increase in combustion with the addition of glucose can be obtained by the present methods of studying these tissues in vitro. This is a constant finding of many observers in the case of the liver, and of the few who have worked on muscle. The latter also does not utilize fructose and these results stand in opposition to those obtained by perfusion experiments. Steinberg ('27) e.g., found that all three tissues, muscle, liver and kidney, utilized both glucose and fructose during perfusion.

There is general agreement that fructose is oxidized directly and easily by kidney. With liver, fructose produces a definite change in the metabolism characterized by a small rise in O_2 uptake and a very considerable rise in CO_2 output. Dickens and Greville, in only three and four experiments with and without fructose failed to find any rise in O_2 although the CO_2 increased.

The lack of response of liver and kidney (also muscle according to Kisch, '33) to galactose is not so surprising from a consideration of the difficulty that the rat experiences with this sugar. Harding, Grant and Glaister ('34) found, 1 hour after feeding rats galactose, 114 mg. per cent unchanged in the liver and 33 mg. per cent in the muscles.

The R.Q. of muscle, as usually found, is higher than that of kidney and liver (between 0.85 and 0.90) and indicates a high but not exclusive carbohydrate metabolism. It is not possible to explain the indifference of excised muscle to added sugars from the experiments at hand.

A consideration of the available data on the metabolism of liver in vitro greatly tempts one to speculate concerning the processes involved. Definite answers, however, are not forthcoming at present. Does the excessive increase in the CO_2 production with fructose mean that the liver burns only a small part of the sugar and converts another small part directly to fat? Excess CO_2 rather than diminished O_2 is to be expected from carbohydrate to fat conversion (Bleibtreu, '01) and

fructose is known to form glycogen more readily than glucose (Cori, '26; Feyder and Pierce, '35). The high R.Q.'s obtained in the intact body after fructose feedings are generally ascribed to a greater fat formation with fructose than with glucose (Deuel, '36).

The liver of rats fasted 24 to 48 hours gives an average R.Q. as low as Gemmill and Holmes ('35) found after 2 to 9 days of fat feeding. Do these low quotients indicate, as those authors suggest that liver, in contrast with other tissues, has the ability to convert fat to carbohydrate? Cori and Shine ('35) believe that the newly formed carbohydrate of liver found by Gemmill came from glycerol but this assumption leaves unexplained an average R.Q. of 0.58. If all the glycerol were converted to glucose, the R.Q. would not be lower than 0.68. At all events, if the low R.Q. is to be interpreted as evidence of conversion, the process must occur in fasting rats as well as in those fed on fat for several days.

In the intact animal are these two processes, carbohydrate to fat and fat to carbohydrate, going on regularly with the possibility of one or the other predominating at any particular time when the animal is killed and the liver removed? Could this be the explanation of R.Q.'s found in the literature for liver ranging from 0.45 to well over 1.0? Such a wide range of values is found for no other tissue.

SUMMARY

With a modified Warburg, measurements were made on kidney and liver slices and on whole diaphragm muscle of oxygen absorption and of respiratory quotient in Ringer's-phosphate solutions and in solutions of Ringer's phosphate to which had been added glucose, fructose or galactose. With kidney and liver both fed rats and animals fasted 24 to 48 hours were used.

Galactose was without influence on the respiration of kidney tissue, glucose increased the O_2 uptake without changing the R.Q. and fructose caused a greater increase in O_2 than did glucose, together with a rise in R.Q. which corresponded exactly to the theoretical rise.

Muscle tissue oxidized neither glucose nor fructose and liver tissue was intermediate between the two. It oxidized neither glucose nor galactose but fructose it did burn, to some extent, as indicated by a small increase in the O_2 consumption and a rise in R.Q. The latter, however, was so much higher than the theoretical rise as to suggest that processes other than oxidation were taking place, e.g., the conversion of some of the fructose to fat.

The possible role of the liver in carbohydrate metabolism is discussed.

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